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MOVING INTO THE FUTURE: RELOCATING THE SOUTH AFRICAN NATIONAL FISH COLLECTION TO A NEW, DEDICATED COLLECTION FACILITY

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Abstract.—The occasion of relocating a collection is an opportunity to improve the collection. The South African Institute for Aquatic Biodiversity, which houses the National Fish Collection, acquired a new, dedicated collection facility in 2007. The curation committee resolved to take advantage of the opportunity of moving the collection to the new facility to improve the state of curation of physical specimens and to edit specimen database records. For this reason, as well as to make the move process as efficient as possible, a custom-designed interface to the specimen database was created. The new interface will remain a useful collection management tool for the foreseeable future.

Introduction

The National Fish Collection of South Africa in Grahamstown is a 60-year-old collection of freshwater, estuarine and marine fishes, mostly from southern Africa, east Africa, the south eastern Atlantic Ocean, the Western Indian Ocean and the Southern Ocean (Skelton 1997, Gon and Skelton 1997). More than 100 countries are represented by about 70,000 specimen-lots in jars of alcohol, about 5,000 large specimens in alcohol-filled tanks, cleared and stained preparations, skeletal preparations and tissue preparations. A large collection of about 20,000 drawings, paintings, 35mm slides, photographs, digital images and radiographs is associated with the specimen collection. The specimen collection and many of the images and illustrations may be browsed online via the SAIAB Information Portal: http://saiab.ac.za/infoportal (also Coetzer 2007).

Gon (1997) recorded the history of marine fish systematics in South Africa. From 1947 the collection in Grahamstown was under the curatorship of Professor J.L.B. Smith of Rhodes University. Seven years after the establishment of the J.L.B. Smith Institute of Ichthyology in 1968 (Collection code: RUSI) Professor Margaret Smith moved the collection into a new building that had been purpose-built for ichthyological research, with a basement to accommodate the collection. In 1999, following 19 yr as a national museum, the Institute became a National Research Facility of the Foundation for Research Development, which soon became the National Research Foundation (NRF). In 2002 the Institute was renamed the South African Institute for Aquatic Biodiversity (abbreviated as SAIAB; also the new collection code). In 2004 the board of the NRF recommended that a new, dedicated collection facility be built, primarily for reasons of occupational health and safety but also to accommodate the rapidly growing collection, which had already outgrown the space in the basement. The new SAIAB Collection Facility was completed towards the end of 2007 (Fig. 1).

In the SAIAB Collection Facility 123 racks of compactorized shelving now hold the 70,000 non-type specimens in jars. The type specimens are stored separately on 10 racks. A rack has five columns and five rows of shelves (Fig. 2). The tanks containing large specimens are supported by standard, static shelving. The whole specimen collection occupies a contiguous space.

In 1998 the University of Alberta Clothing and Textiles Collection reported the use of a database to plan a move, keep track of the move and to provide information to facilitate access to physical objects (Lambert et al. 1998). Late in 2005 the SAIAB collection-move

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Figure 1. The new SAIAB Collection Facility, which was completed in December 2007.

team, coordinated by the second author, began packing jars into numbered boxes that would later be moved to the SAIAB Collection Facility. The development and use of a custom-designed Microsoft Access front-end, connected to the Specify specimen database, is described. The Inventory, Curation and Reshelving (ICR) front-end, developed by the first author, allowed us to relocate the specimen collection efficiently and by exploiting a rare opportunity to improve the physical condition of, and update the database records of, as many specimen-lots as was possible. The ICR front-end will be an indispensable collection management tool long after the last box of jars was unpacked onto the shelves in the new collection facility in May 2008.

The Specimen Database

The catalog of the National Fish Collection was digitized in 1990 (Gon and Wertlen 1996) and in 2001 the database was migrated from a custom-designed Advanced Pick database to Specify 3.0 (Kansas University Natural History Museum and Biodiversity Research Center) (A. Bentley, pers. comm.). When packing for relocation began in 2005, about 90% of the collection was cataloged, with at most about 10,000 specimen-lots still uncataloged. With all staff occupied in packing and unpacking the collection from late 2005 to early 2008, however, the backlog of uncataloged material rapidly grew by a further 10,000 lots. On average, SAIAB researchers continue to collect about 2,000 new specimen-lots per year. Several large collections had been made during recent cruises in the Western Indian Ocean, which considerably increased this average. The fact that most of the shelved collection was cataloged when relocation began made the specimen database that much more useful during the relocation.

Figure 2. The compactorized shelving in the SAIAB Collection Facility.

By the time relocation started the Specify 5.2.1 front-end was in use. This has since been upgraded to Specify 5.2.3. The font-end connects to a Microsoft SQL Server 2000 back-end on a Microsoft Windows Server 2003 machine. About 15 users in the Institute connect to the server from machines running Microsoft Windows XP Professional, via a

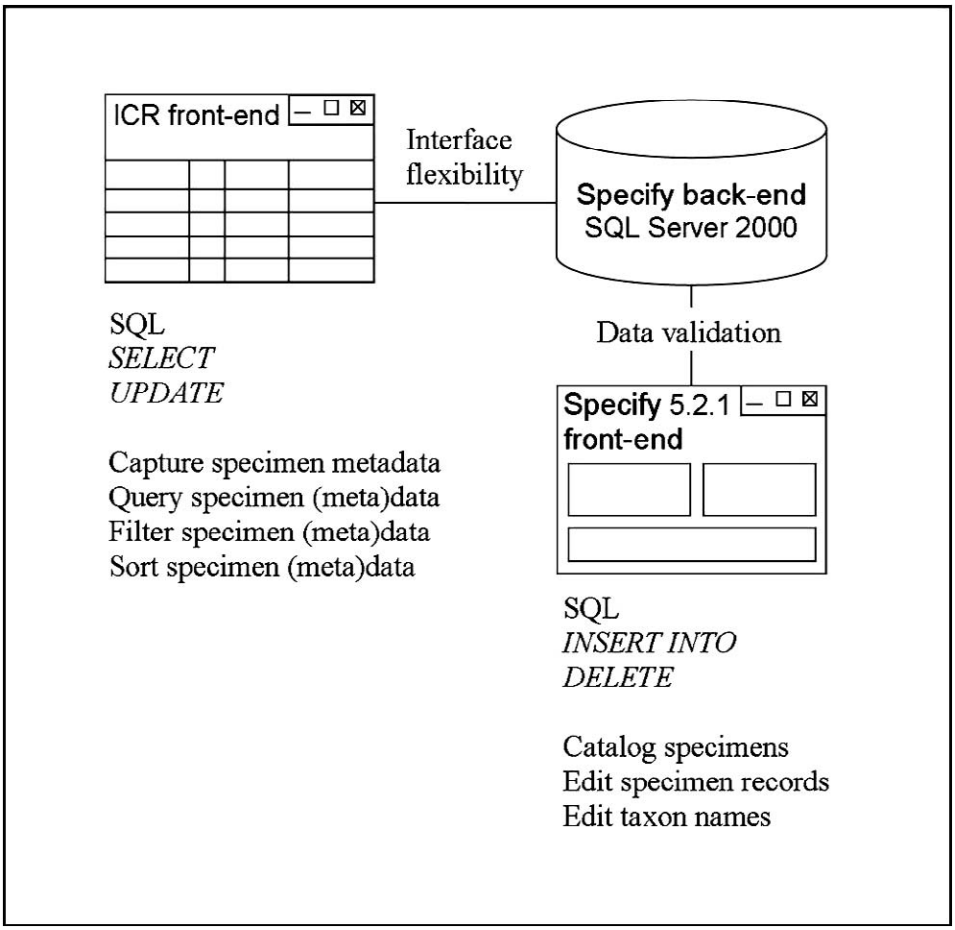

Figure 3. Schematic diagram to show the ICR front-end, which offers useful user-forms, connecting to the Specify back-end. The Specify front-end continues to be used as usual for data validation in specimen cataloging and editing, and loan processing, and for opportunistic querying and label printing.

conventional cable network. Specify has not only proved to be an indispensable tool, but is easy to use and is backed by excellent, free support.

The Objectives of Using the Inventory, Curation and Reshelving Front-end

Collection staff continued to catalog specimen-lots and edit specimen records and taxon names, using the Specify front-end for the strict data validation of Specify. The Specify front-end was also used for opportunistic queries. A Microsoft Access Project (.adp file format), called the Inventory, Curation and Reshelving front-end (ICR), was developed for specific workflows (Fig. 3). Initially, these workflows were exclusively defined by the need to relocate the collection (e.g., recording the numbers of boxes into which jars were packed, or recording the kind of label contained in each jar). Because processes such as label reprinting would continue long after relocation, possibly for the next decade, the ICR front-end was developed into a new tool that could be used indefinitely in parallel to the Specify front-end. Curation and collection management tasks that are not directly related to the collection relocation, such as recalling overdue

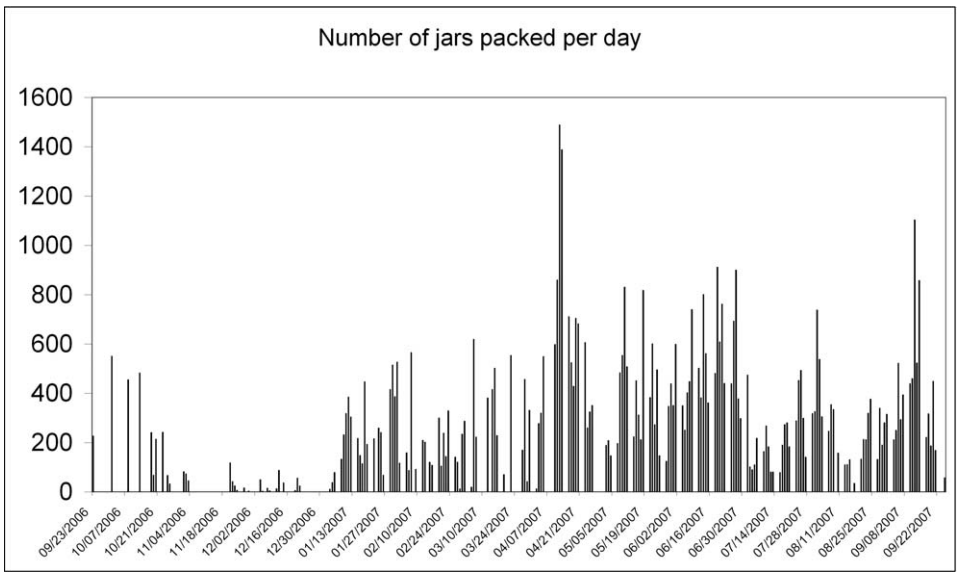

Figure 4. A histogram to show the variability in the daily rate of packing. This was also used to predict when packing would be complete.

loans, are increasingly being managed through the use of the ICR front-end (Fig. 6) but always by using the ICR as a front-end to data captured in Specify, as has been done since 2001.

The main purpose of the ICR front-end was to read current information from the Specify database and display it in custom-designed user-forms (Fig. 5). Specify user-forms are also customisable, but complex Microsoft Access forms can be developed more quickly and more easily than Specify forms, and they incorporate more useful functions for end-users. This is especially true during user requirements gathering, when users are discovering and describing how the information they want to see should best be displayed. While editing in Microsoft Access forms can be done in 'continuous' forms (i.e., forms with rows, resembling spreadsheets, e.g., Fig. 6), editing in Specify 5.2.3 can only be done in 'detail view' (i.e., only a single record is visible). Viewing and editing sequential information was a prerequisite for efficiently packing and unpacking the specimen jars and for auditing tank contents.

Writing to the Specify database from the ICR front-end was also an important function, but this was limited to updating previously unused fields in existing rows in Specify tables, and did not involve inserting rows into, or deleting rows from, any Specify tables. The custom-designed user-forms in the ICR front-end:

1) satisfied the needs of packers and unpackers to see particular fields of information displayed in particular ways;
2) allowed quick and easy navigation between different forms, which could be kept open simultaneously;
3) allowed information to be sorted, filtered, printed or exported easily, by functions and buttons that are familiar to users, and
4) could display the complex new sequence in which jars were unpacked onto the new shelves, a function that neither a Specify query nor an individual could perform.

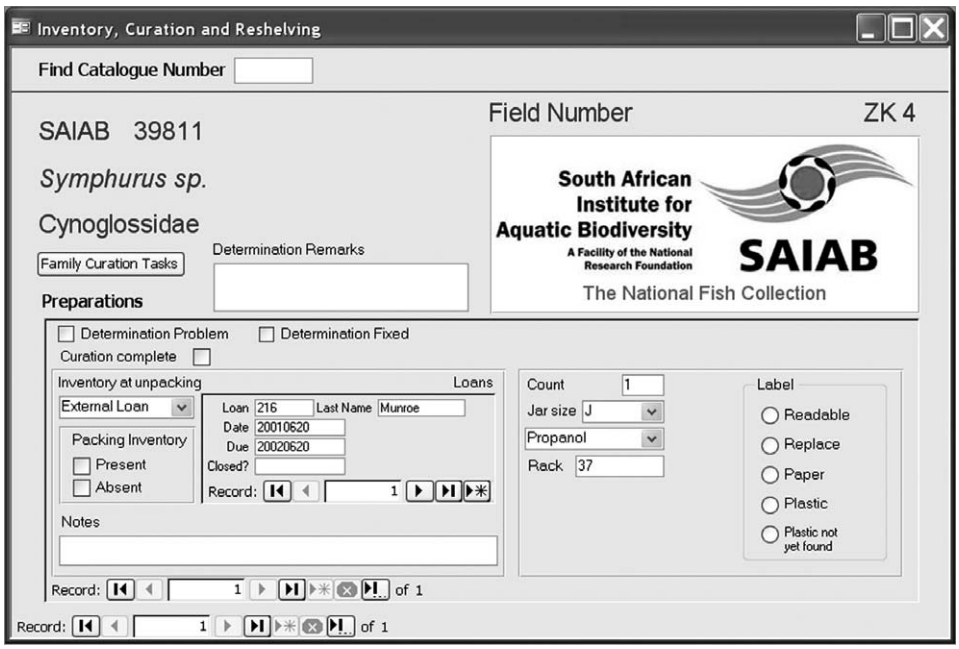

Figure 5. An example of a 'single' (detail view) user-form in the ICR front-end, which includes specimen loan details.

Rather than profiling the collection on the basis of shelves (Favret et al. 2007), the coordinator of the collection move decided to use the opportunity to handle every specimen-lot during the relocation of the collection so as to:

1) conduct an inventory of the 75,000 specimens in jars and tanks;
2) record basic information about the condition of each jar and tank specimen for later remedial curation (the jar size and label type were recorded in every instance);
3) measure unmeasured tank specimens and verify their tank numbers, and
4) perform emergency remedial curation mid-move where possible (e.g., replace rusty jar lids).

The fish collection is of a size that allowed at least some information to be recorded about each jar or tank-specimen. The result is a list of unfinished, jar-specific curation tasks, which can be addressed on a continuous basis.

Manipulation of information using the new tool allowed us to:

1) monitor the rate of packing and unpacking according to weekly targets, and diagnose the causes of inefficiency (Fig. 4);
2) project, by regression analysis, the amount of shelf space that would be required for each family (or even each large species) in 10 years' time;
3) re-order the jar collection on the new shelves according to a complex sequence to facilitate searching, and re-order the tanks containing large specimens;
4) fit all the jars onto the shelves while leaving enough space within and between racks for future growth, and
5) keep a current map of the jars and tanks, at the scale of the shelf in the type collection and at the scale of the rack for the non-types.

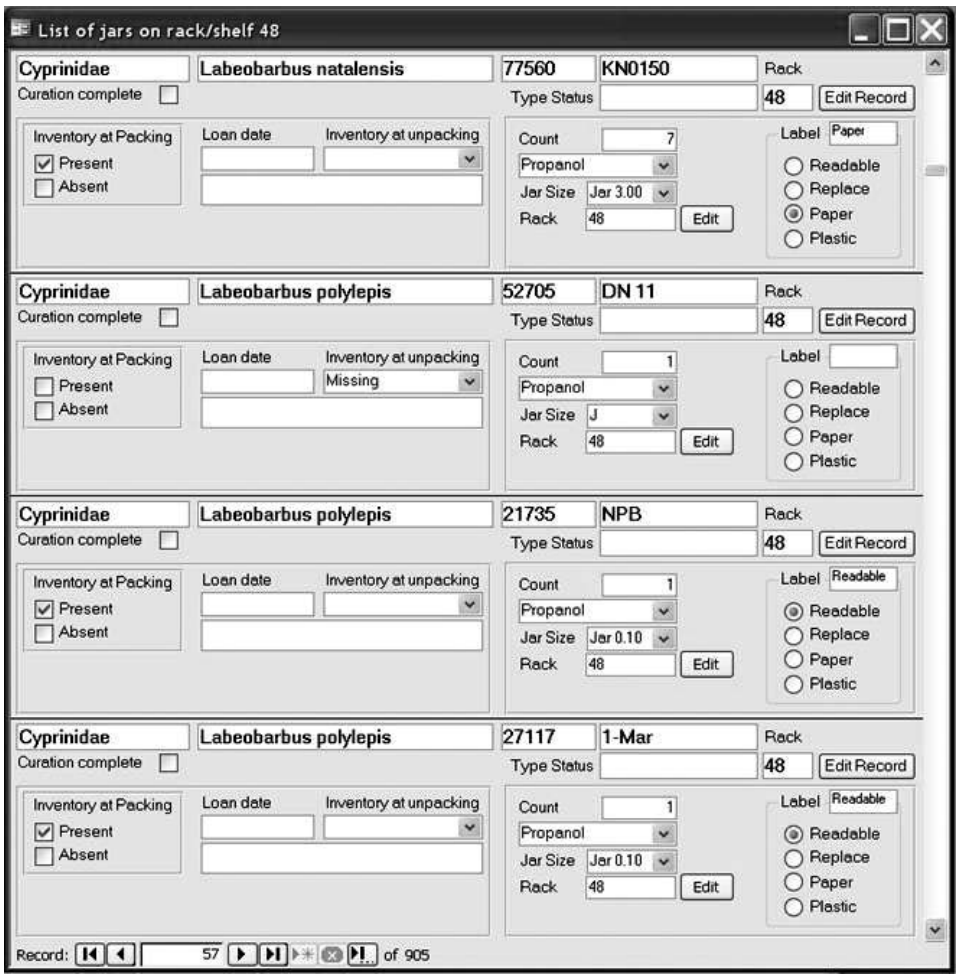

Figure 6. An example of a 'continuous' (spreadsheet-like) user-form in the ICR front-end, in which specimen-lots appear in the new shelf sequence.

Remedial Curation, Sorting and Packing

Preparators topped up alcohol, replaced rusty lids and inadequate jars, sealed 0.10 L and 0.25 L jars with parafilm, and then arranged the jars of a particular family or group of small families into family-, genus-, species-, jar-size, and catalog-number order.

Packers then packed these prepared jars into numbered boxes, in the sequence described above, using the ICR front-end to record the following specimen metadata:

1) whether the jar was present or absent (later analysis would resolve which absent specimens had been borrowed);
2) the jar size (jar size had not been recorded before in the history of the collection);
3) the type of label i.e., whether the label needed immediate replacement, was old but legible, an oven-baked paper label or a new, heat-transfer, plastic label;
4) the box number, and
5) any notes for later remedial curation, such as whether the jar seemed to contain a mixed lot.

By recording the box number that each jar (identified by its catalog number) was packed into, we were assured that the contents of the box would be recorded. This meant that boxes didn't need to be moved immediately after they had been filled, but could instead await later relocation. This allowed us to start packing before the collection facility's foundation had been laid, so as to be ready to move in as soon as the building was commissioned. It also meant that we could change the taxon names of boxed specimens at our convenience without affecting the retrieval of those specimens. For example, a number of family names had to be changed before the jars could be unpacked from their boxes. To find a series of specimens we only needed to arrange the boxes in numerical order and generate a list of taxon names and catalog numbers and their associated box numbers. It took the packing teams about 18 mo to pack all 70,000 jars into boxes.

Unpacking

The packed boxes were transferred to the new collection facility, and placed, still full of jars, onto the new shelves, roughly in the places where they would be unpacked. Unpackers then generated lists of required box numbers and retrieved those boxes from a line of numerically arranged boxes on the floor. Unpacking required one person to read the catalog number of each jar in the sequence and another to find the jar in the box and place it on the shelf. Because the jars were already in sequence in the boxes, unpacking the jars in sequence onto the new shelves was quick. The entire unpacking process took only four months. The sequence of jars was changed from a phylogenetic arrangement of families on the old shelves to an alphabetical arrangement of families on the new shelves. Within families jars were sorted by genus- and species name, then by jar size (to increase shelf-packing efficiency and to make it easier to see all the jars on a shelf) and finally by catalog number for each jar size. Specimens determined as '[Family]' were unpacked onto the shelves at the beginning of a particular family's allocated space, those determined as '[*Genus*] sp.' were placed before the first species of a genus, and those determined as '[*Genus*] *cf.* [*species*]' were placed before the species with which they are compared. While this is a complex sorting exercise for a person to carry out, it is simple to design as a query in Microsoft Access. This single function was reason enough to design and use a separate Microsoft Access interface.

Some of the curation problems discovered while moving the collection included:

1) uncataloged specimens and mixed lots;
2) mis-shelved specimens;
3) non-type specimens in the type collection and vice versa;
4) jars that had incorrect label details or contained labels belonging to other jars, and
5) incorrectly spelled generic or specific names in the Specify database.

The last item above was typical of a curation problem that could only be discovered by digitally sorting a list of names that was read as a box was unpacked. When a name did not appear in the sorted list, but a jar in sequence in the box needed to be placed on the shelf, it meant that there was probably a spelling error in the taxon name field of the specimen record, or a similar error that excluded the record from the sequence, which could be recorded (using the ICR front-end) and corrected at a later stage.

The Collection Map

Whereas the arrangement of the fish collection has been depicted schematically for a long time, we developed a new kind of map that can be updated (e.g., biannually or

annually) to reflect the current rack number (non-types) or shelf number (types) of any jar. Whenever the last jar on a rack or shelf changes (due to jars being inserted before the last jar and pushing it off the rack or shelf, or due to jars being inserted after the last jar), a note is made of the catalog number of the jar that is now in the last position. The shelf sequence is generated by a query in the ICR front-end. By updating, through the ICR front-end but in the specimen database, the rack numbers and shelf numbers of all the jars that are in the last position on each non-type rack (at most 123 numbers) and type shelf (at most 250 numbers), the rack and shelf numbers of all jars between successive 'last-position jars' can be updated automatically.

Discussion

There have been at least two stock-taking exercises in the National Fish Collection in the last decade. Because information from these stock-taking events was recorded on printed lists, it is not as easily accessible as the information collected during the 2006 inventory. The fact that the ICR front-end displays current information from the Specify database is its most important advantage. For example, we can capture, in Specify, the as yet undigitized loans issued before 1995, recall all overdue loans and update the 2006 inventory information accordingly, using the ICR front-end. The collection map (which is updated by queries in the ICR front-end) will make specimen retrieval quick, easy and efficient, especially when a large batch of specimens needs to be fetched. The collection map will also allow us to easily plan for, and communicate about, the movement of jars, caused by batch acquisitions or rapid growth, for example. Importantly, we are now able to execute future curation tasks rack by rack (non-types) or shelf by shelf (types), knowing precisely how many, and which, jars are on each rack or shelf, and the sequence in which they are packed. In the near future we will replace most of our small jars, which are inadequate, with new jars, and we will change all propanol to ethanol. The ICR front-end will no doubt be indispensable during this work. Finally, Microsoft Access can be a useful interface development tool, for viewing specimen data in specific ways, when user requirements are not met by the interface of a custom specimen database.

Conclusion

Our experience of moving, reordering, stock-taking, and trouble-shooting this relatively large collection was made easier and more efficient by enabling the collection move team to manipulate information in the specimen database by using the specially developed ICR front-end. The ICR front-end also allowed us to update the specimen database with information that we will use to improve the condition of many specimen-lots. Many errors in the specimen database would not have been found were it not for the way we systematically read each database record by comparing it to a physical collection object. We plan to perform a similar inventory and improvement exercise with the collection of about 5,000 jars containing tissue specimens preserved in ethanol. These need to be subsampled and the subsamples need to be frozen. In the process we hope to correct a number of known anomalies in the database records.

We conclude by reflecting on the history of curation and management of the National Fish Collection. On 9 October 1985 Paul H. Skelton convened a meeting to discuss curation and, specifically, the need for a Curation Committee. The committee would 'consider all matters dealing with curation … to provide advice and guidance on collection management and use.' Among the various terms of reference were curatorial policy and practice, the investigation of different preservatives and preservation

regimens, the compilation of a curation bibliography, budgeting, fund-raising and the acquisition of suitable specimen containers. But the first item for consideration was 'computerization of the collection'. The curation committee survives to this day, as does a healthy cataloging backlog and the never-ending search for the perfect cataloger.

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The National Fish Collection has been built, managed and cared for by many people, too numerous to acknowledge individually. We wish to thank Roger Bills, Wouter Holleman (who also suggested improvements to the manuscript), Bafo Konqobe, Jerraleigh Kruger, Hilton Lewis, Mark Lisher, Bernard Mackenzie, Mandilakhe Mkalipi, Vusi Mthombeni, Margie Shaw and Sally Terry for moving the collection into the future.

Literature Cited

Coetzer, W. 2007. Desktop access to biodiversity. Quest 3(2):12–13.

Favret, C., K.S. Cummings, R.J. McGinley, E.J. Heske, K.P. Johnson, C.A. Phillips, L.R. Phillippe, M.E. Retzer, C.A. Taylor, and M.J. Wetzel. 2007. Profiling natural history collections: A method for quantitative and comparative health assessment. Collection Forum 22(1–2):53–65.

Gon, O. 1997. Fifty years of marine fish systematics at the J.L.B. Smith Institute of Ichthyology. Pp. 45–78 in *The J.L.B. Smith Institute of Ichthyology—50 Years* (P.H. Skelton and J.R.E. Lutjeharms, eds.). Transactions of the Royal Society of South Africa, Vol. 51.

Gon, O. and P.H. Skelton. 1997. A history of the fish collections of South Africa. Pp. 133–168 in *Collection Building in Ichthyology and Herpetology* (T.W. Pietsch and W.D. Anderson, eds.). The American Society of Ichthyologists and Herpetologists.

Gon, O. and R. Wertlen. 1996. Fishnet, a computerized database management system for the national fish collection at the J.L.B. Smith Institute of Ichthyology. South African Journal of Science 92(3):117–121.

Lambert, A., S. McLean, and J. Whittome. A collection on the move. In: Resource Manual: Moving Collections Workshop, 13th Annual Meeting of the Society for the Preservation of Natural History Collections, University of Alberta, Edmonton, Canada, 25–30 May 1998.

SAIAB Information Portal. 2007. http://saiab.ac.za/infoportal (5 December 2008).

Skelton, P.H. 1997. A historical review of the taxonomy and biogeography of freshwater fishes in South Africa—the past 50 years. Pp. 91–114 in *The J.L.B. Smith Institute of Ichthyology—50 Years* (P.H. Skelton and J.R.E. Lutjeharms, eds.). Transactions of the Royal Society of South Africa, Vol. 51.

DETECTION AND ENUMERATION OF POTENTIALLY ZOONOTIC BACTERIA ON A CETACEAN SPECIMEN DURING MACERATION

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Abstract.—Maceration is a technique traditionally used by many museums and other institutions wishing to prepare the bones of vertebrate specimens for study and exhibition. At the Natural History Museum (NHM), London, maceration is frequently used by the Department of Zoology, when preparing cetacean remains recovered as part of its National Cetacean Strandings Project. Specifically, sections of mandible containing teeth were taken from carcasses of beached animals, with the intention of removing the teeth for thin-sectioning and age determination.

In recent years, health and safety concerns have been raised regarding the zoonotic exposure risks to staff carrying out the maceration. It is because of these concerns that the NHM has undertaken this study. In this investigation, potentially zoonotic bacteria on a dolphin's jawbone were detected and enumerated. The detection of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium perfringens* and *Escherichia coli* was based on colonial morphology and the effects of growth on microbiological nutrient media. These bacterial species were also enumerated by culturing diluted jawbone sample suspensions on diagnostic nutrient media.

Introduction

Maceration previously carried out at the Natural History Museum involved the manual removal of soft tissues from carcasses using surgical instruments such as knives and scalpels. The bones were then placed in containers of water and were either kept at ambient temperature, or heated to 37°C (98.6°F) to allow certain bacteria to grow and help breakdown residual soft tissue. This technique was also favoured as a non-chemical, non-invasive and non-destructive process, preserving DNA, stable isotopes and other materials inside the bones which are of use in subsequent scientific research. This maceration technique was carried out on the jawbone of cetaceans. The maceration of larger vertebrate specimens in plain water has long since ceased because of health and safety considerations, so alternative techniques such as the use of biological enzymes in solution are being investigated.

Marine mammals such as dolphins can be infected with, or be healthy carriers of, zoonotic pathogens, i.e., disease agents transmissible from animals to humans (Mazet et al. 2004). This is, therefore, a serious health and safety consideration during the maceration procedure when carried out on the cetacean specimens in the Museum. The person(s) carrying out this procedure may be exposed to potentially zoonotic micro-organisms (bacteria, viruses, fungi etc.). Infection can result, for example, by inhaling aerosols containing these microbes or via cuts and lacerations from dissecting instruments.

There have been many reports documenting isolations of potentially zoonotic bacterial pathogens from dolphins. Chan et al. (2001) examined fifteen captive dolphins over a 7-year period and found the following organisms as representing greater than 2% isolates: *Proteus mirabilis* (6.5%), *Staphylococcus aureus* (2.4%) and *Pseudomonas aeruginosa* (2.1%).

Asper and Odell (1980) sampled 26 wild dolphins from the East Coast of Florida and found *Escherichia coli*, *Staphylococcus epidermidis*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* most common in blowhole samples. Also, Buck et al. (2006) found that 30% of all the bottlenose dolphins that were sampled had species of *Staphylococcus* and

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suggested that given the increasing significance of staphylococci in human infection, their recovery from dolphins should receive additional attention in terms of more careful screening of isolates from marine mammals.

In the UK, the Zoological Society of London has isolated the following potentially zoonotic bacterial pathogens from cetaceans: *Brucella* spp., *Salmonella* sp. (*Salmonella typhimurium* 0412:a), *Vibrio* species (e.g., *V. parahaemolyticus*, *V. damsela*). *Clostridium* species (e.g., *C. botulinum*, *C. perfringens*), beta-haemolytic *Streptococcus* species (e.g., Lancefield Group L), *Erysipelothrix rhusiopathiae* and *Edwardsiella tarda*. *Brucella* spp. have seldom been isolated at the Zoological Society of London. They are difficult to grow in culture and, therefore, could easily be missed. However, government laboratories in Scotland and Cornwall have isolated *Brucella* spp. from numerous porpoises and some other species (e.g., bottlenose dolphins) (P. Jepson pers. comm.).

The aim of this project is to detect primarily the presence of four groups of bacteria on the dolphin's jawbone. They are as follows: (1) *Streptococcus* species (2) *Staphylococcus* species (3) *Clostridium* species and (4) coliforms e.g., *Escherichia coli*. The colonial morphology of these bacteria and the effects of their growth on diagnostic media were used to detect their presence.

Materials and Methods

Maceration of the Jawbone of the Dolphin

The specimen used for this study was the jawbone from an unidentified species of dolphin found beached and very decomposed on the Hampshire coast in June 2002.

Firstly, maceration of the dolphin's jawbone was required. It was cut into four pieces, each piece approximately 3 cm in length. Two pieces of the jawbone were autoclaved at 121°C for 15 min at 15 p.s.i. following which each was immersed in 100 ml of autoclaved tap water in a plastic container. One piece was kept at room temperature (21°C, 69.8°F) and the other in a 37°C water bath (control samples). The other two pieces of non-autoclaved jawbone were immersed in non-autoclaved tap water and kept at the same two temperatures (test samples). The above procedures were carried out in a fume cupboard. After one week maceration was completed, that is, the flesh could be easily removed from the bone. After maceration the test sample at 21°C was a pale brown cloudy liquid and the test sample at 37°C was a bluish-green cloudy liquid. The control sample at 21°C was a clear liquid and the control sample at 37°C was a pale brown, but not cloudy, liquid.

Detection of Potentially Zoonotic Bacteria on the Jawbone of the Dolphin

Four types of diagnostic media (Mannitol Salt Agar, Blood Agar, Cooked Meat Medium and MacConkey Agar) were inoculated with a small amount of the jawbone sample suspension using a sterile wire loop. A fifth medium, Reinforced Clostridial Agar, was inoculated by mixing the sample with the molten agar medium. Inoculation of all diagnostic media was carried out in a Class I Hybrid Recirculating type Safety Cabinet. Replicates of all inoculated media were incubated at 37°C and 44°C (111.2°F) for 24 hr, except for the inoculated Mannitol Salt Agar and Cooked Meat media, which were incubated at 35°C (95°F) for 3 days and up to 21 days respectively.

Enumeration of the Potentially Zoonotic Bacteria

Serial dilutions were carried out on the jawbone sample suspensions with quarter-strength Ringer Solution and 100 µl of the diluted sample was then spread on the

Table 1. Description of bacterial colonies on diagnostic media after 24 hr incubation at 35°C and 37°C.

Sample	MaCA 37°C incubation	BA 37°C incubation	RCA 37°C incubation	MSA 35°C incubation	CMM 35°C incubation
Test sample at 37°C	Profuse growth. Slight greening of the pink medium. Red and a few green-brown and pale pink colonies.	Dense, profuse growth. Bluish-green colonies	Slight break-up of the solid medium as a result of gas production. Colonies were greyish-white and approx. 1.0 mm–1.5 mm in diameter.	Small yellow colonies approx. 0.5 mm in diameter. Yellowing of the pink medium around the colonies.	Turbid growth. Acid and gas production was observed after 3 days incubation. There was reddened protein in the meat particles.
Test sample at Room Temperature (21°C)	Round red colonies approx. 0.5 mm–1.5 mm in diameter. Very slight greening of the medium.	Dense, profuse growth. Bluish-green colonies. Slight haemolysis.	There were several cracks in the solid agar medium as a result of gas production. Colonies were 1.0 mm–1.5 mm in diameter.	Same as above	Same as above

MaCA = MacConkey Agar.
CMM= Cooked Meat Medium.
BA = Blood Agar.
RCA = Reinforced Clostridial Agar.
MSA = Mannitol Salt Agar.

diagnostic media. In the case of *Clostridia* spp, however, 1 ml of the serial dilution was transferred in triplicate into sterilised flattened bottles. Freshly prepared Reinforced Clostridial Agar medium was then cooled to 50°C (122°F) and without shaking, 15 ml of the diluted sample was added to each bottle. The medium was sealed immediately with melted sterile paraffin and allowed to set in a water bath at 15°C (59°F) (Bridson 1990).

All inoculated media were incubated at 37°C and 44°C, except for the enumeration of *Staphylococcus aureus*, where the Mannitol Salt Agar medium was incubated at 35°C for three days.

Results

The results of detection of bacteria on diagnostic media are shown in Tables 1 and 2. There were many red colonies and a few pale-pink and green-brown colonies on the MacConkey Agar media incubated at 37°C. However, there were only red colonies on the MacConkey Agar media incubated at 44°C. The ability to grow on this medium indicates the presence of a presumptive coliform.

All the colonies on the Mannitol Salt Agar media were surrounded by bright yellow zones as described in Table 1, indicating the presence of pathogenic staphylococci. There was good microbial growth on all the Reinforced Clostridial Agar media incubated at both 37°C and 44°C, also in the Cooked Meat Media which were incubated at 35°C. Growth on these two media indicates the presence of an anaerobe such as a *Clostridium* species. The bluish-green colonies on all the Blood Agar media as described in Tables 1 and 2 indicates the presence of a *Pseudomonas* species.

Table 2. Description of bacterial colonies on diagnostic media after 24 hr incubation at 44°C.

Sample	MaCA	BA	RCA
Test sample at (37°C)	Discrete red colonies approx 0.5 mm–1.0 mm in diameter. Very slight greening of the medium.	Profuse growth. Bluish-green colonies. Slight haemolysis.	Difficult to observe individual colonies. No gas production in the medium.
Test sample at Room Temperature (21°C)	Colonies were smooth, glossy, translucent and red in colour. They were approx. 2.0 mm–2.5 mm in diameter.	Profuse growth. Bluish-green colonies.	No gas production in the medium. Difficult to detect discrete colonies.

MaCA = MacConkey Agar.
BA = Blood Agar.
RCA = Reinforced Clostridial Agar.

Results of the enumeration of the four bacterial species detected on the jawbone of the dolphin are shown in Table 3. Generally, the total number of bacteria was greater in the jawbone suspensions kept at 21°C than those kept at 37°C.

Discussion

In this study, four species of bacteria were detected on the jawbone of the dolphin. It was possible to detect these bacteria by their colonial morphology and the effects of their growth on diagnostic microbiological nutrient media.

These nutrient media are commonly used for the detection and enumeration of potentially pathogenic bacteria. The nutrient components of such culture media are carefully selected to recover the required spectrum of organisms in the sample e.g., coliforms, anaerobes etc. A general purpose medium such as blood agar in its various forms will often contain mixtures of peptones to ensure that peptides of different variety are available for the great majority of organisms likely to be present. Whole blood can be used to detect haemolytic enzymes produced by some organisms.

The Blood Agar medium was used initially in this investigation to detect *Streptococcus* species. It is a medium made from the combination of Blood Agar Base No. 2 and 10% defribinated horse blood. Blood Agar Base No. 2 has nutritional properties suitable for the cultivation of fastidious pathogens and other micro-organisms, and the defribinated horse blood allows the detection of an organism such as a *Streptococcus* species with haemolytic properties. Although this group of bacteria was not present on the jawbone sample, the medium allowed the detection of *Pseudomonas aeruginosa*. This organism produces a distinctive bluish-green diffusible pigment in the medium and also slight haemolysis of the medium. It was observed during the maceration process at 37°C that the production of the bluish-green pigment was greatly enhanced at this temperature

Table 3. Enumeration of the bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*) on the dolphin jawbone samples.

Bacteria	Mean number of organisms/ml of triplicate sample suspensions at 21°C	Mean number of organisms/ml of triplicate sample suspensions at 37°C
Pseudomonas aeruginosa	4.7×10^5	3.5×10^6
Staphylococcus aureus	3.5×10^6	3.9×10^5
Clostridium perfringens	1.8×10^7	7.8×10^6
Escherichia coli	8.2×10^7	7.6×10^6

resulting in the jawbone sample suspension having this particular colour (production of this pigment at 37°C is characteristic of *Pseudomonas aeruginosa*). The sample suspension at 21°C was not bluish-green during or after maceration. The flesh was easily removed from the jawbone after seven days in tap water at 21°C and 37°C, so the higher temperature did not seem to encourage the growth of micro-organisms that accelerate the breakdown of tissue.

The addition of coloured indicator substances is a very effective way of detecting fermentation of specific carbohydrates by bacteria in a culture medium. Such compounds (e.g., neutral red) should change colour distinctly and rapidly at critical pH values. A chemical selective agent such as bile salts can also be added at a specific concentration to suppress the growth of unwanted micro-organisms in a polymicrobial sample (Bridson 1990).

The MacConkey Agar medium used in this study contains both neutral red and bile salts and the ability to grow on this medium indicates the presence of a presumptive coliform(s) on the jawbone sample. In this case, it is most likely to be the lactose-fermenting *Escherichia coli*, which produces red colonies on this medium. Furthermore there was very good growth at 44°C and pathogenic *Escherichia coli* grows well at this particular temperature.

The selective medium, Mannitol Salt Agar was used for the detection of presumptive pathogenic staphylococci. Most other bacteria are inhibited by the high salt concentration with the exception of some halophilic marine organisms. The predominant growth on this medium indicated the presence of *Staphylococcus aureus*, which is potentially pathogenic to humans. Pathogenic staphylococci produce colonies that are surrounded by bright yellow zones, whereas, non-pathogenic staphylococci produce colonies with reddish-purple zones.

Finally two nutrient media, Reinforced Clostridial Agar and and Cooked Meat Medium were used to detect anaerobes, especially *Clostridium* species. Good microbial growth and gas production on the Reinforced Clostridial Agar medium indicated the presence of *Clostridium perfringens*. Cooked Meat Medium is prepared from heart tissue and has the ability to initiate bacterial growth from very small inocula. The addition of glucose to the formulation allows heavy growth of anaerobic bacteria in a short time and leads to a more rapid identification of important anaerobes such as *Clostridium perfringens*. This organism rapidly produces acid and gas from glucose fermentation in the Cooked Meat Medium. The meat becomes reddened, but there is no digestion of the meat. The organism also seem to produce foul-smelling hydrogen sulphide. These effects on the Cooked Meat Medium is characteristic of *Clostridium perfringens*.

The results of the enumeration of the bacterial species investigation showed that abundances were greater at 21°C than at 37°C for three species. It is possible that the pieces of jawbone at 21°C had more flesh, hence more bacterial growth. However, it must be emphasised that the infective dose does not have to be high to acquire a bacterial infection and in a few cases, just 100 cells can cause an infection in humans. Finally, as expected, none of the four groups of potentially pathogenic bacteria were detected in the control samples (sterilised jawbone in sterile water at 21°C and 37°C).

Any of the four species of bacteria that were detected may have been part of the normal flora of the dolphin, caused infections in the dolphin or were on the jawbone as a result of handling by humans or simply originated from the surrounding environment of the dolphin. All, however, can cause diseases in human. *Pseudomonas aeruginosa* has been implicated in pulmonary infections, burn wound infections, urinary tract infections and

gastroenteritis. The anaerobic, spore-forming, *Clostridium perfringens* can cause soft tissue infections, food poisoning and enteritis necroticans.

The ubiquitous *Staphylococcus aureus* has been implicated in a range of infections including post-operative wound infections, pneumonia and food poisoning. Finally, *Escherichia coli* is an opportunistic pathogen and some strains can cause gastroenteritis, urinary tract infections and septicaemia (Murray et al. 1994).

Recommendations for Safe Handling of Cetacean Specimens During and after Maceration

When handling cetacean specimens, it is important that all cuts and abrasions on the hands and face of the preparator(s) are covered with water-proof dressings as these would prevent the micro-organisms from entering 'breaks' in the skin. Very few microbes can penetrate intact skin. Suitable protective gloves, preferably latex gloves, must be worn and if necessary, 'double-gloving', so that the microbes do not get onto the hands, which can be excellent vehicles for spreading any contamination. A suitable disposable mask and safety glasses must be worn, so that droplets or aerosols containing microbes from the cetaceans cannot get into the body by inhalation or through the mucous membranes of the eyes.

Maceration of the specimens must be carried out at 21°C or room temperature in a fume cupboard or in a similar environment. The fume cupboard is a major means of controlling the user's exposure to chemicals and other air-borne hazardous materials such as aerosols containing micro-organisms. When the maceration is completed, any waste material must be subjected to a destructive cycle (134°C, 273°F for 45 min) in an autoclave. The autoclaved material must then be incinerated and the surfaces where the maceration work has been carried out must be cleaned with a disinfectant so that microbes in any spillages will be killed.

Procedures that produce aerosols must be avoided when handling the specimens outside the fume cupboard, as these aerosols may be infectious. Finally, hands must be washed thoroughly before eating, drinking and smoking, and keep other exposed skin clean.

Conclusions

The results have shown that potentially zoonotic bacteria are present on fleshed cetacean specimens. Safe handling is therefore necessary during and after the maceration process to prevent acquiring infections. Generally, the use of personal protective equipment, dedicated areas for preparation, strict health and safety protocols for working with and safe disposal of tissues are required to minimise the risks of infection.

Literature Cited

Asper, E.D. and D.K. Odell. 1980. Tursiops truncatus *Studies*. H/SWI Technical Report No. 80-122 to National Marine Fisheries Service. Hubbs/Sea World Research Institute, Orlando, Florida.

Bridson, E.Y. 1990. *The Oxoid Manual,* 6th ed. Unipath Ltd, England. 339 pp.

Buck, J.D., R.S. Wells, H.L. Rhinehart, and L.J. Hansen. 2006. Aerobic micro-organisms associated with free-ranging dolphins in coastal Gulf of Mexico and Atlantic Ocean waters. Journal of Wildlife Diseases 42(3):536–544.

Chan, O.S., J. Mukerjee, R.E. Kinoshita, and C.S. Yuen. 2001. Microbial flora of blowhole samples in captive bottlenose dolphins (*Tursiops truncatus aduncas-type*) in Hong Kong, 1993–2000. *Proceedings of the 32nd Annual Conference of the International Association for Aquatic Animal Medicine*. Tampa, Florida, pp. 24–25.

Jepson, P.D. 2006. Personal communication. Co-ordinator, Marine Mammals Strandings Research, Zoological Society of London, Regent's Park, London NW1 4RY.

Mazet, J.A.K., T.D. Hunt, and M.H. Ziccardi. 2004. Assessment of the risk of zoonotic disease transmission to marine mammal workers and the public: survey of occupational risks in Final Report, Wildlife Centre, University of California, pp. 3–6.

Murray, P.R., G.S. Kobayashi, M.A. Pfaller, and K.S. Rosenthal. 1994. *Medical Microbiology,* 2nd ed. Mosby Europe. 755 pp.

A NEW APPROACH TO STABILIZE THE PH IN FLUID-PRESERVED NATURAL HISTORY COLLECTIONS

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Abstract.—The maintenance of a neutral pH is, besides avoiding evaporation and decreasing alcohol concentration, the third and most complicated aspect regarding the curation of preservation fluids in natural history collections. Both the measurement and the subsequent adjustment of the pH inside the specimen jars are fraught with considerable theoretical and methodological difficulties. We propose a new approach to avoid the problem altogether by stabilizing the desired pH with substrate-bound ion-exchange materials, e.g., an ampholyte provided with positively and negatively charged groups in form of pellets, sheets or sticks. Alternatively a combination of separate acidic and basic ion-exchange substrates could be employed.

INTRODUCTION

Curatorial problems with fluid preserved natural history collections deal with various aspects such as containers, lids and labels but most importantly with the preservation fluid itself. Ethanol, which constitutes today the preferred medium in natural history collections, has been in use as a preservation fluid since the 17th century (Down 1989, Moore 1998). The respective methodology is nonetheless still based almost exclusively on empirical knowledge instead of scientific expertise (Moore 1998, Waller and Simmons 2003).

The situation may seem simple at first glance: a jar containing the specimens, some labels, a certain volume of air at the top, and – of course – the preservation fluid. However, the specimen jar with its contents constitutes a more or less closed system that is subjected to a variety of changes during years of storage, due to interaction with its environment (especially evaporation) as well as interaction amongst the various contents, such as leaching or oxidation (von Endt 1994, Marte et al. 2003, Oberer 2008). As a result, the preservation fluid may soon change its properties regarding volume, alcohol concentration, solved substances, as well as pH. These changes, in turn, may lead to serious damage or decomposition of the specimens. It is therefore necessary to monitor the collection and reconstitute the desired properties by curatorial measures regularly.

While it is theoretically possible to monitor not only the fluid level and colour but also its alcohol concentration with little effort today (e.g., with a portable density meter), the monitoring and maintenance of the pH is still problematic in theory as well as in practice. In this paper we address specifically the problems of maintaining the desired pH.

STATEMENT OF PROBLEM

Values below pH 6.5 can cause decalcification of bony structures and otoliths, hardening of specimens, as well as protein embrittlement and dissociation (Thede 1996, Gotte and Reynolds 1997, Moore 1998, van Guelpen 1999, Hargrave et al. 2005). Alkaline conditions substantially above pH 7.0 on the other hand cause clearing of soft tissues, as proteins and lipids are leached from the specimens (Taylor 1977, Dingerkus 1982, Stoddard 1989, Gotte and Reynolds 1997, Hargrave et al. 2005).

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Recent surveys reveal that the pH in natural history collections may indeed reach well into problematic ranges on the acidic as well as the alkaline side. For example, Dingerkus (1982) found values ranging from pH 2 to pH 9 in an ichthyological collection. Other surveys resulted in ranges of pH 5.4–8.2 (Simmons and Waller 1993) and pH 4.5–7, pH 5–6 or pH 5.2–7.6 (Waller and Simmons 2003) for herpetological collections and pH 5–7 (Cato 1990) and 4.8–8.9 (Palmer 1996) for mammal collections, depending on the applied measuring method.

An extensive screening across a wide range of taxa in the wet collections of two large European museums (Naturhistorisches Museum Basel, Zoologische Staatssammlung München, Kotrba et al. in preparation) revealed a range of pH 4.5–9.5 for both institutions, with 14% of the samples ranging at pH 6 or smaller and 14% ranging at pH 8 or larger. This study also showed that the pH of samples a priori classified as ''probably OK'' is not necessarily better than that of samples classified as ''probably problematic.''

Although the enumerated facts clearly show that problems with the pH in fluid preserved natural history collections exist and cannot be neglected, no study has yet come up with suggestions for practicable standard procedures for the maintenance of a desired (neutral) pH.

Open problems remain regarding the measurement and interpretation of the actual pH in the preservation fluid, which is generally ethanol with a water content of 30% or less (tap or deionised) and the addition of some denaturizing agents. The low water content as well as contaminations with proteins, lipids and other substances seriously limit the accuracy of pH measurements (Waller and Simmons 2003, Sound and Becker 2007).

A second, even larger challenge is the reconstitution of the desired pH, if unacceptable values are detected. Standard curatorial measures, i.e., topping up with alcohol to restore alcohol levels and concentration, generally have no effect to the improvement of the pH (Cato 1990, Kotrba et al. in preparation). Titration is unfeasible for various reasons starting with the above-mentioned difficulties with the pH measurement and the related time effort. Also it is usually not possible to appropriately stir the fluid without disturbing the specimens. Moreover titration generally involves the risk of precipitations forming deposits on the specimens.

At present, the recommended procedure is to completely exchange the entire preservation fluid repeatedly (e.g., Dingerkus 1982). However, this procedure may considerably disturb the specimens and lead to additional leaching.

Dealing with the accounted difficulties surpasses the physico-chemical expertise of the average curator. Appropriate standard procedures are not available and their development would require the help of a specialized chemist or analyst. Therefore the most common curatorial approach today is to ignore the problem altogether.

New Approach

It is here suggested to circumvent the related problems by stabilizing the desired pH in the preservation fluid from the very start by buffering the system with the help of a solid ion-exchange material.

The use of buffering agents is already commonplace with respect to formalin fixation of biological specimens and the storage of such formalin fixed material. While some authors suggest the use of sodium borate, i.e., borax (Miller 1952, Taylor 1967) others explicitly advise against this practice (Dingerkus 1982, Taylor 1977). Gotte and Reynolds (1997) recommend the use of a sodium phosphate monobasic / sodium phosphate dibasic buffer, and Taylor (1977) suggests the use of ground limestone.

The physico-chemical advances of the last decades allow us to propose a different approach for stabilizing the pH in ethanol based preservation fluids with the help of a substrate-bound ion-exchange material such as a substrate-bound ampholyte. This concept is similar to a method proposed by Eugster and Righetti (1993) for long term pH control in solutions for medical purposes.

A substrate-bound ampholyte is a polymeric substrate provided with positively and negatively charged groups. Appropriate negative immobile groups are carboxyl groups, sulphuric acid groups or phosphoric acid groups and the positive immobile groups are generally various types of amino groups. If the suggested ampholytic material is not available, alternatively a combination of an acidic and an alkaline ion-exchange substrate, each in a separate batch, can be applied.

The polymeric substrate may be shaped into pellets, sheets or sticks. It may be composed of any polymeric material which is permeable and chemical resistant to the preservation fluid. Unfortunately some commonly used substrates such as polystyrene are unsuitable for our purpose, because they are not resistant to methyl ethyl ketone. The latter is commonly used as a denaturizing agent in alcohol and is known to cause swelling and decomposition of many synthetic polymeric materials. There are, however, good long-term experiences with paper labels in historical collections, therefore cellulose as substrate for the ion-exchange material might be considered as an alternative and is already known as a possible material (e.g., Wade and Brown 1979).

A further step would be to combine the ion-exchange substrate with a colour pH indicator that reveals exhaustion of its capacity (such as that proposed by Härtel and Schmidt 1981). By this measure a simple inspection would be sufficient to recognize all jars with deficient pH.

As compared to the addition of dissolved or powdery buffering agents directly to the preservation fluid, the proposed method would have the following advantages:

1) The buffering agent and its reaction products do not interact directly with the stored specimens, e.g., by forming insoluble deposits on their surface.
2) Pellets contained in a net, sheets, or sticks can be easily retrieved from the specimen jar without disturbing the specimens.
3) The ion-exchange material can be regenerated by rinsing with acidic or alkaline solute and subsequently reused.
4) If a combination of separate acidic and alkaline ion-exchange substrates is applied, then only the exhausted ion-exchange substrate has to be replaced, once the direction of pH deviation in a specific specimen jar is detected.

Conclusion

The proposed concept for the maintenance of a stable pH in natural history wet collections now needs to be put into concrete terms, especially regarding the chemical and physical aspects. Suitable ion-exchange materials have to be identified, produced and tested with respect to the achievable buffer capacity and long term stability in preservation fluid. Moreover studies involving the preservation of standardized specimens from laboratory cultures such as lab mice, cockroaches, etc. are needed to provide quantitative information on the kinetics of pH changes in buffered and unbuffered specimen jars. Such studies could simultaneously show to which extent pH changes occur and actually cause damage to the preserved specimens and/or containers and which buffer capacity over time and which economic effort is necessary to avoid said pH changes.

Such studies are in the interest of the museum community rather than the individual researcher. We strongly encourage the societies involved with natural history conservation issues such as SPNHC, ICOM-CC, or Synthesys NA C to launch and support respective research projects.

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Literature Cited

Cato, P.S. 1990. Characteristics of a collection of fluid-preserved mammals and implications for collection management. Collection Forum 6:53–64.

Dingerkus, G. 1982. Preliminary observations on acidification of alcohol in museum specimens jars. Curation Newsletter 5:1–3.

Down, R. 1989. "Old" preservative methods. Pp. 33–38 in *Conservation of Natural History Specimens: Spirit Collections* (C.V. Horie, ed.). The University of Manchester, Manchester, England. viii 115 pp.

Eugster, C. and P.G. Righetti. 1993. Process for stabilising the pH of aqueous feed solutions and for absorbing and buffering urine and wound secretions. Patent document: EP 0425590 B1.

Gotte, S.W. and R.P. Reynolds. 1997. Observations of the effects of alcohol vs. formalin storage of amphibian larvae. Oral presentation at the workshop "Preservation and curation of early life history stages of fishes, amphibians, and reptiles" at the meeting of the American Society of Ichthyologists and Herpetologists in Seattle, Washington, 26 June–2 July 1997. (http://www.pwrc.usgs.gov/resshow/reynld1rs/amphlarv.htm)

Hargrave, C.W., K.G. Sutherland, E. Marsh-Matthews, and W.J. Matthews. 2005. Multiple interacting factors affect pH in museum storage solutions. Collection Forum 19:23–31.

Härtel, M. and E. Schmidt. 1981. Schwachsaure Indikator-Kationenaustauscher und Verfahren zu ihrer Herstellung. Patent document: DE 2157275 C2.

Marte, F., C. Solazzo, D. von Endt, D. Erhardt, and C.S. Tumosa. 2003. The stability of natural history specimens in fluid-preserved collections. 6th International Congress, Cultural Heritage: Context and Conservation in Havana, Cuba, 14–18 April 2003 [CD post print]. (http://www.si.edu/MCI/downloads/articles/nhcstability.pdf)

Miller, W.R. 1952. Treated formalin as a permanent preservative. Turtox News 30:178–179.

Moore, S. 1998. Fluid preservation. Pp. 92–132 in *Care & Conservation of Natural History Collections* (D. Carter and A.K. Walker, eds.). Butterworth-Heinemann, Oxford, England. 256 pp.

Oberer, C. 2008. Neues vom Präparateglas: eine unerwartete Wende. Oral presentation at the kickoff workshop "KUR—Programme for Conservation and Restauration of the mobile Cultural Heritage" in Berlin, Germany, 16 August 2008.

Palmer, L. 1996. Assessing preservation fluids in a wet collection: How important is container style? Natural History Conservation 10:10–12.

Sound, I. and H. Becker. 2007. Grenzen der pH-Messung in nichtwässrigen Lösungsmitteln. LaborPraxis November 2007:44–45.

Stoddard, R.W. 1989. Fixatives and preservatives: Their effects on tissue. Pp. 1–32 in *Conservation of Natural History Specimens: Spirit Collections* (C.V. Horie, ed.). The University of Manchester, Manchester, England. viii 115 pp.

Taylor, W.R. 1967. An enzyme method of clearing and staining small vertebrates. Proceedings of the United States National Museum 122(3596):1–17.

Taylor, W.R. 1977. Observations on specimen fixation. Proceedings of the Biological Society of Washington 90:753–763.

Thede, C.E. 1996. Survey of a fluid-preserved anatomical collection. Natural History Conservation 10:12–17.

Van Guelpen, L. 1999. Larval fish preservation: Ethanol acidity from Byron Weston Resistall label paper. Curation Newsletter 12:12–14.

Von Endt, D.W. 1994. Spirit collections: A preliminary analysis of some organic materials found in the storage fluids of mammals. Collection Forum 10:10–19.

Wade, K.O. and J.H. Brown. 1979. Thermally reversible amphoteric ion exchange resins of improved demineralization capacity. Patent document: US 4139499 A.

Waller, R. and J.E. Simmons. 2003. An exploratory assessment of the state of a fluid-preserved herpetological collection. Collection Forum 18:1–37.

THE INHERENT LEVELS OF ARSENIC AND MERCURY IN ARTIFACT MATERIALS

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Abstract.—Interest in metal-based pesticides present in museum artifacts increased due to the Native American Graves Protection and Repatriation Act (NAGPRA), under which many Native American artifacts are being repatriated for cultural uses that may involve direct contact with humans. Mercury and arsenic are of greatest concern due to high persistence. They have been added as pesticides and preservatives over the past ~100 years, but in general, use has not been documented. An important aspect that needs to be considered in the analysis of an artifact for metal contamination is that many artifacts contain mercury and arsenic due to their inherent composition.

Understanding exogenous versus endogenous sources of arsenic and mercurial contamination is imperative in the assessment of toxin levels on artifacts in museums as well as for the development of solutions and treatments for removing those toxins. The natural and anthropologic sources of mercury and arsenic are considered, and a literature review of the level of these toxins, which may be inherent in materials used to make Native American artifacts is presented. The baseline arsenic and mercury inherent in artifact materials before their storage or treatment using mercurial or arsenic preservatives by collectors or museums may be as high as 20 ppm for hair, feathers or skin due to bonding with the sulfhydryl groups in these materials. Wood and other plant materials should not have levels in excess of 500 ppb unless exposed to high concentration soils or water. These values should be used only as a general guideline in the complex analytical and toxicological decision making process required for the repatriation of objects.

INTRODUCTION

Natural history specimens and ethnographic artifacts have been historically treated with arsenic and mercury salts in order to protect them from insects. A study done by the Canadian Institute of Conservation on collections from five museums indicated that approximately eighty percent of the natural history specimens contained arsenic and/ or mercury (Sirois 2001). This has created an environmental concern for museum workers and the public who may be exposed to these toxins. Also, museums are frequently asked to return sacred objects under the Native American Graves Protection and Repatriation Act. Now artifacts that are otherwise well contained and rarely handled are being returned to tribes for culturally appropriate use. Several museums, including the Arizona State Museum in collaboration with various departments in the College of Engineering at the University of Arizona, are in the process of developing and evaluating cleansing and extraction solutions to remove the arsenicals and mercury compounds. The artifacts of concern are typically made from feathers, fur, hair, wood, animal skin or plant materials such as reed or grass. While analytical methods for the determination of mercury and arsenic have been well defined, there has been no comprehensive literature review of the inherent levels of these elements in natural objects (Palmer 2001). The understanding of naturally occurring levels of arsenic and mercury in the materials of interest is critical in differentiating whether an object is contaminated and whether treatment should be limited to surface cleaning or extended to extractive leaching of subsurface contaminants. The information is also useful in determining the appropriate end-point for cleansing treatments. The purpose of this

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paper is to summarize data from the literature on typical natural levels of arsenic and mercury found on materials.

Sources of Arsenic

Arsenic is the 52nd most abundant element and is distributed throughout the earth's crust. Major arsenic-containing minerals include arsenopyrite (iron arsenic sulfide), realgar (arsenic monosulfide) and orpiment (arsenic trisulfide). Others include cobalite (cobalt iron arsenic sulfide), enargite (copper arsenic sulfide), proustite (silver arsenic sulfide), tennantite (copper arsenic sulfide) in addition to native arsenic. Higher than average arsenic concentration is associated with sandstones, shales and coal. Clays and sedimentary iron and manganese oxides can also be rich in arsenic (Focazio et al. 2000). Arsenic in the lithosphere can come from emissions that occur as a by-product of mining and electricity generation. Mining and smelting of nonferrous ores contribute to arsenic emissions, as arsenic is usually associated with the sulfides of nonferrous deposits. Combustion of coal for the generation of electricity also is a source of arsenic.

Arsenic is naturally introduced into water when minerals and ores such as iron, cobalt and lead are dissolved by water. Arsenic can also enter the ground water through leach fields, slag heaps or from air deposition from smelters.

Historical uses of arsenic include the use of pigments such as copper arsenates (known as Paris green, emerald green, Scheele's green, Schweinfurt green), arsenic sulfide (known as realgar and orpiment) in fabrics, wallpapers and paints. Hundreds of arsenic greens were manufactured and used until the turn of the 20th century when it was linked to many deaths. It was also a common component in Victorian fly papers, pesticides, and rat poisons, a common preservative in taxidermy, and medicinal additive for the treatment of illnesses such as syphilis, leprosy and the skin disease yaws.

Modern day uses of arsenic compounds include the use of calcium arsenate, lead arsenate, cuprous arsenite, sodium arsenite and arsenic trioxide for control of insects in agriculture, to control the ripening of fruit and as a cotton defoliant. Arsenic is used commercially as an alloying agent, and the semiconductor and photonics industries use arsenic for ion implant of silicon-based devices and GaAs for semiconductor devices. Arsenic also was widely used as a wood preservative although the wood-treating industry made a decision to eliminate all arsenical wood preservatives from residential use by the end of 2003 (Kwon et al. 2004). Organic arsenicals are widely used for weed control, pesticides, in poultry farming and to enhance milk production in cows. The organic forms are thought to be less harmful to health, as the body can readily eliminate them (Carter et al. 2003).

Inorganic forms of arsenic include trivalent arsenite (As [III]) and pentavalent arsenate (As [V]). Inorganic arsenicals were largely used in the past as insecticides to control specific insects but are no longer in use. They do, however, persist in fields and orchards where they were applied (Belluck et al. 2003), and they remain on museum artifacts where there was widespread use in pest control (Odegaard and Sadongei 2001).

Sources of Mercury

Mercury is a liquid at ambient temperatures and pressure with a density roughly 13.5 times that of water. Mercuric (II) sulfide, also known as cinnabar (vermilion or orange red in color) is the most common naturally occurring mineral containing mercury. Organic rich shales tend to have high levels of mercury as do coal and crude oil. Bituminous coal in the United States typically contains 1 to 25 parts per billion (ppb)

mercury while anthracite coals contain 1,200 to 2,700 ppb. California crude oil contains 1,900 to 21,000 ppb of mercury. Other minerals that commonly contain mercury include schwatzite, sphalerite and wurtzite (Pecore 1970).

Mercury is found in the lithosphere due to its tendency to vaporize. Atmospheric levels are a few ng/m^3 air. Mercury concentrations are higher over mines with levels of 20,000 ng/m^3 reported over mercury mines, 1,500 ng/m^3 reported over precious metal mines and 20 ng/m^3 reported over copper mines (Swain et al. 1992). The burning of fossil fuels and coal based electrical plants contribute heavily to the atmospheric burden of mercury.

Mercury was used by 100 AD for amalgamation extraction of gold and the mercury bearing mineral cinnabar was used as a red pigment by both indigenous persons and industrialized cultures. Mercuric nitrate was used in the process of converting pelts to felt in the 18th and 19th centuries until it was discovered to be the cause of eraditic behavior patterns and health issues in hatters (Martin and Kite 2003). Mercury is still used in the amalgamation of metals for extractive metallurgy today. Other more recent uses include: the manufacture of caustic soda and chlorine; in paints as a mildew retardant, as antiseptics and disinfectants, and in industrial control instruments.

Levels of Arsenic and Mercury in Materials

Hair or Fur

Native American artifacts that include hair or fur in their design are typically made from human hair, animal hair or animal fur. Arsenic and mercury deposit in the sulfur-rich hair from the blood stream or from external sources such as water or the atmosphere. Hair analysis is a technique commonly used to assess exposure to these elements. Many hair analysis studies have been done on mammals and humans under both natural conditions and after exposure to high levels of toxins. The accuracy and repeatability of different analysis techniques for determining trace elemental levels in hair has long been a source of debate. A more recent study done on samples sent to different laboratories showed that the laboratories had significantly different results (Seidel et al. 2001). The differences can be due to the measurement capability of the analytical equipment or differences in the sample preparation technique so that data presented in the literature should only be considered as an estimate.

Table 1 summarizes the levels of arsenic and mercury found in the hair and fur of animals.

One study on the mercury levels in the hair of Alaskan reindeer indicated a mean level of 55.3 ppb mercury for free-range reindeer. Reindeer are vegetarians and eat forage that includes lichens. Lichens accumulate atmospheric contaminants and could be a source of mercury (Duffy et al. 2005). Analysis of fur samples done to determine the mercury burdens of furbearers in Wisconsin - such as beaver, muskrat, raccoon, mink and river otter - showed that the highest fur levels for mink were 41.2 ppm and 63.2 ppm for otter. Mean values were as follows: beaver 0.03 ppm, muskrat 0.06 ppm, red fox 0.55 ppm, raccoon 3.79 ppm, mink 7.61 ppm, and river otter 6.47 ppm (Sheffy and St. Amat 1982). Similar mercury levels were found in Georgia, where mink mercury levels ranged from 2.3 to 17.3 ppm and otter mercury levels ranged from 9.3 to 67.9 ppm. (Cumbie 1975).

The mercury levels of hair in muskrats and mink were analyzed in a study done on the Oakridge Reservation in Tennessee. The herbivorous muskrats were found to have hair mercury concentrations ranging from 0.05 to 22.6 mg/kg (equivalent to parts per million) while the carnivorous mink showed mercury levels ranging from 8 to 14.7 mg/kg for mink

Table 1. The levels of arsenic and mercury found in animal hair and fur.

Animal	Arsenic and mercury level	Analytical technique	Location/environment	Reference
Sheep wool	Hg: 48 ppb As: 51 ppb, 570 ppb	CV-AAS	Germany. Hg:control As: sheep fed on contaminated soil, sheep raised in urban area	Gebel et al. 1996.
Reindeer	Hg: 55.3 ppb	CV- Atomic Fluorescence	Alaska- free range	Duffy et al. 2005.
Elk	As:18 ppm	Hydride Generation: AAS	Yellowstone National Park, USA. Exposed to arsenic laden thermal waters	Kocar et al. 2004.
Beaver, muskrat, mink, raccoon	Hg: Mean levels of 0.03 to 6.47 ppm. Maximum level up to 63.2 ppm.	Flameless AAS	21 counties from Wisconsin, USA.	Sheffy et al. 1982.
Otter, mink	Hg: 2.3 to 67.9 ppm	Flameless AAS	Lower coastal plain of Georgia, USA.	Cumbie, 1975.
Otter	Hg: 2.2 to 18.8 ppm	AAS	Prince William Sound, Alaska, USA. Uncontaminated site.	Ben-David et al. 2001.
Otter	Hg: 4 to 200 ppm	Hydride Generation ICP-MS	South Central Ontario Canada	Evans et al. 1998.
Muskrats, mink	Hg: up to 104 ppm	CV-AAS	Tennessee, USA. Historically contaminated rivers.	Stevens et al. 1997.

along the Bear Creek and up to a mean value of 104 for mink living along the East Fork Poplar Creek, both of which have been historically contaminated with mercury (Stevens et al. 1997).

In a study done on river otter , the mercury detected in the fur ranged from 2.2 to 18.8 parts per million (ppm) (Ben-David et al. 2001). In another study, the mercury levels in otter fur in Ontario, Canada, were measured at 4 to 20 ppm (Evans et al. 1998).

Wool from sheep grazing on grounds contaminated with mercury and arsenic showed significantly elevated levels of mercury (0.107 mg/kg versus 0.048 mg/kg for control sheep, $P < 0.001$). The arsenic content was 0.051 mg/kg, versus 0.57 for sheep grazing in an urban reference area. No definitive anthropogenic source of exposure was presented (Gebel et al. 1996).

An extensive study done on elk exposed to arsenic from the geothermal waters in Yellowstone National Park, USA, showed that the elk had arsenic levels of up to 18 mg/kg in hair samples (Kocar et al. 2004).

In summary, river animals with fur tended to have ppm levels of mercury while the hair of animals tended to be in the ppb range. This is most likely due to the length of time that the fur is exposed to the water containing mercury. In contrast sheep and reindeer tended to have ppb levels of contamination. The arsenic levels increased 10 fold for sheep raised in an urban area with no known source of arsenic and elk living near geothermal water containing arsenic had levels of 18 ppm arsenic. This suggest that in addition to ingestion, external sources of contamination such as sorption from vapors may be a factor as well as the hydrophobic nature of each mammals hair or fur and the tendency of the hair to be coated with substances such as lanolin in sheep hair which prevent sorption of contaminants.

The data summarized in Table 2 indicates that human hair may contain up to 6 ppm of mercury and typical levels of arsenic are under 3 ppm when abnormal exposure has not

Table 2. The levels of arsenic and mercury found in human hair.

Arsenic or mercury levels found in hair	Analytical technique/equipment	Location/ environment	Reference
0.04 to 1.04 ppm As	Hydride atomic absorption spectroscopy	Egypt / Non-occupational population	Saad and Hassanien, 2001
Up to 0.2 ppm arsenic, increasing with age up to 12 mo.	Neutron activation	Canada / Infants up to 12 mo	Gibson and Gage, 1982
14.1 ppm As on average	Energy Dispersive X-ray Fluorescence(EDXRF). Detection limit 2.5 ppm.	Bangladesh / Drinking water with 100ppb or greater arsenic	Ali and Tarafdar, 2002
0.37 to 14.1 ppm As depending on and correlated to exposure level to chemicals	Neutron activation	Nigeria / Worker population exposed to wood preservatives	Ndiokwere, 1985
7–106 ppm As	Hydride atomic absorption spectroscopy	Workers in a semiconductor plant using arsenic	dePeyster et al. 1995
Hg level means: China – 1.7 ppm Indonesia – 3.1 ppm Japan – 4.6 ppm	Cold vapor atomic absorption spectroscopy (CV-AAS)	China, Indonesia, and Japan	Feng et al. 1998
5.6–6.1 ppm mean Hg	CV-AAS	Singapore / Multicultural population	Foo et al. 1998
0.07 to 0.96 ppm Hg	CA-AAS	Sweden / Pregnant women	Oskarsson et al. 1994
<0.06 to 1.7 ppm Hg	CV-AAS	Germany / School -age (8–9) children	Pesch et al. 2002

occurred. Levels above 3 ppm may be caused by excessive exposure. For example, the mean arsenic hair levels found in a study done in Bangladesh were found to be 14.1 ppm. This elevated level is due to consumption of water from tube wells where arsenic concentrations exceed 100 ppb (Ali and Tarafdar 2002). Like humans, we would expect the levels of arsenic in the hair of other mammals exposed to high concentrations of arsenic in drinking water to be greater. There are some areas where high arsenic concentrations naturally occur in the surface and ground waters due to the weathering of local minerals and soils. The Verde Valley in Arizona is one such notable location (Foust et al. 2004).

Drinking water is not the only significant factor that can increase arsenic levels in mammals. Saad and Hassanien (2001) showed a significant increase in arsenic hair levels from cigarette smoking and eating fish for healthy non-occupational Egyptians. In a study done on various groups of workers in a semiconductors plant, factors such as sex, tap water consumption and dietary habits were significant contributors of arsenic and mercury levels in hair while the persons job type, even if exposure to arsenic was more probable, did not show a significant effect. Overall levels in this group were high with a range of 7 to 106 ppm (dePeyster and Silvers 1995). The smoking of molasses tobacco increased the arsenic levels. Likewise, several significant contributors increase mercury levels in human hair. Pesch et al. (2002) found a positive correlation between the smoking habits of parents, fish consumption and the number of teeth with amalgams on the hair mercury levels of school children in Germany. The correlation between freshwater or saltwater fish consumption and mercury levels in the hair of pregnant women and

Sweden was found to be positive (Oskarsson et al. 1994). Foo et al. (1998) found that fish consumption, sex and ethnicity were all factors in elevated mercury levels in hair. Males had higher levels and the Chinese population had higher levels than the Malay or East Indian populations in Singapore. The ethnic variability is thought to be due to diet and the use of traditional medicines, which may contain mercury.

Thus we may hypothesize that objects exposed to the smoke from burning tobacco or other plants containing arsenic and mercury may have had accumulated arsenic and mercury deposits on them. While we would expect the baseline for mercury in horsehair to be comparable to that of reindeer at about 50 ppb, it possibly could be as high as 20 ppm or greater if exposure to a contaminated water source occurred. Levels as high as 20 ppm of arsenic could also be due to environmental exposure.

Feathers

Bird feathers have been analyzed for heavy metal pollution as a bio-indicator of the contamination levels in the environment. Arsenic and mercury accumulates in feathers due to endogenous deposition from the food that birds eat or through exogenous conditions in which emissions from atmospheric conditions or the environment cause the toxins to deposit. Toxins also can be deposited onto bird feathers by secretions from the uropygial gland during preening (Veerle et al. 2004). The uropygial glands of Swedish birds were analyzed and found to contain mean values of 1.24 ppm mercury and 0.96 ppm arsenic, indicating that this is a valid pathway for deposition (Goede and Bruin 1984). A bird's feathers contains more than 70 percent of the body burden of mercury (Monteiro et al. 1998). There is a statistically significant correlation between how high up on the food chain that the bird's food is and the level of mercury found in the feathers of the bird. Higher trophic birds that eat foods such as fish, tend to have higher mercury levels than birds that are vegetarian (Burger and Gochfeld 1996). By comparing new growth feathers to old feathers on the same bird, it was shown that the mercury content in feathers is strictly endogenous while arsenic concentrations appear to be primarily exogenous (Veerle et al. 2004).

Although many techniques have been used for elemental analysis, the technique most commonly used for analysis of mercury in feathers is the cold vapor atomic absorption spectroscopy technique. Graphite furnace atomic absorption is most commonly used for detection of arsenic. Detection levels by these techniques have been noted to be 2 ppb for mercury and 0.09 ppb for arsenic (Burger and Gochfeld 2000). The feathers are commonly washed vigorously in water and acetone prior to an acid digestion and analysis. Mercury is tightly bound to the keratins in feathers so that the washing procedure does not leach the mercury from the feathers (Appelquist et al. 1984).

Table 3 shows the levels of arsenic and mercury in bird feathers indicated by various studies done around the world. The levels not only vary by trophic level and species, they also vary within the bird. Secondary feathers have been shown to have higher concentrations of mercury than breast and tertiary feathers (Burger and Gochfeld 1997). Mercury levels in the environment have increased substantially since the beginning of the industrial revolution (Montiero et al. 1997). One study, repeated on the same species of bird at the same site after 10 years, indicated that the mercury levels had more than doubled (Becker et al. 2002).

The indication is that there is a wide range of mercury and arsenic inherent in feathers. Levels on the order of 1 to 20 ppm can be expected. Environmental locales exposed to anthropologic sources of arsenic such as coal burning power plants and metallurgical

Table 3. The levels of arsenic and mercury found in bird feathers reported as the range of measured values.

Arsenic or mercury levels found in feathers	Analytical technique/ equipment	Species	Location/ environment	Reference
0.6 to 9.2 ppm Hg in several body feather types	Flow Injection Mercury System / FIMS 400 Perkin Elmer	13 species of seabirds	Bird Island, South Georgia (54°03′S,38°36′W) / Marine environment	Becker et al. 2002.
0.06 to 1.1 ppm Hg Sampled and analyzed over a variety of different feather types	CV-AAS	little and intermediate cattle egrets	Bali and Sulawesi, Indonesia / Marine environment	Burger and Gochfeld, 1997.
0.2 to 5.3 ppm Hg in breast feathers	Cold Vapor atomic absorption spectroscopy (CV-AAS)	Double-breasted cormorant, Black-crowned night heron, Franklin's gull	Agassiz National Wildlife Refuge Minnesota, USA / Freshwater marshes and wetlands	Burger and Gochfeld, 1996.
0.05 to 0.5 ppm As in Breast feathers. 0.5 to 19.7 ppm Hg in breast feathers.	Hg: CV-AAS As: Graphite furnace atomic absorption Perkin Elmer 5000	12 species of seabirds	Mid Atoll (28°15′ N, 177°20′W) Northern Pacific Ocean Marine environment	Burger and Gochfeld, 2000.
2.1 to 22.3 ppm Hg in breast feathers	CV-AAS / Perkin-Elmer Mercury Analyzer	Seabirds	Azores (36°to30°N, 25° to31° W) / Marine environment	Monteiro et al. 1998.
Mercury: 0.25–5.42 ppm 0.83–3.41 ppm <MDL-0.70ppm <MDL-0.46ppm in body feathers (MDL = 0.01ppm)	CV-AAS	Bonelli's eagles Jay Domest. pigeon Partridge	Southwest Portugal / Upland mountains	Palma et al. 2005.
0.57 to 0.65 ppm Hg in tail feathers. 1.56 ppm As in new growth tail feathers. 7.13 to 9.16 ppm As in old growth tail feathers.	Axial inductively coupled plasma-mass spectrometer (ICP-MS, Varian Ultramass 700)	Great tits	Antwerp, Belgium/ Industrial area of city	Veerle et al. 2004

Note: MDL is the minimum detection limit.

plants create highly increased baseline levels of arsenic and/or mercury in local bird feathers. Due to its more volatile nature, mercury is infiltrating the atmosphere and waters of the planet, creating a detectable shift in baseline mercury levels removed from the point-of-source. This may have an impact on the levels of mercury found on older versus newer artifacts.

Skin

Skins used for artifacts come from the hides of many animals. The animal hides used most often by Native American Indians are deer, antelope, buffalo and cattle. Accumulation of arsenic in skin from the lithosphere or hydrosphere occurs due to the affinity of arsenic for the sulfhydryl groups in the skin (Hostynek et al. 1993). Kwon et al. (2004) have shown that arsenic from chromated copper arsenate-treated wood accumulates on the hands of school children.

Very little information was found on natural arsenic and mercury levels in skin. Only one study was found that tested natural arsenic skin levels of a mammal. Elk foraging near arsenic-laden waters in Yellowstone Park, USA, had arsenic skin levels of 0 to 3.5 ppm (Kocar et al. 2004). Studies done to monitor the levels of arsenic and mercury in the skin of river snakes indicated levels of 86 ± 27 ppb arsenic and 159 ± 23 ppb mercury (Burger et al. 2007a). In another study, the levels of arsenic were 0.15 to 140 ppb and mercury 90.5 to 917 ppb (Campbell et al. 2005).

The majority of the literature related to levels of arsenic and mercury in skin were controlled absorption studies. These comparisons are included as well to give an indication of how actual preservation treatments may have impacted skin artifacts.

Dutiewicz (1977) analyzed the penetration of 0.01, 0.1, and 0.2M solutions of sodium arsenate into rat skin. The arsenic penetrated into the rat and accumulated, as evidenced by increasing levels in the spleen and liver. This indicates that arsenic exposure causes penetration throughout the skin and into the body and is not merely isolated to the surface of the skin. Abdel-Rahmen et al. (2005) showed that 44. 6 percent of 2 μg arsenic applied as H_3AsO_4 and 66.3 percent of 187 ng mercury applied as mercuric chloride penetrated into pig skins. Combining the toxins with soils significantly reduced the amount of toxin penetration. It was concluded from the study that the skin acts as a reservoir for arsenic and mercury and that arsenic and mercury can be released systemically over time.

In vitro studies on Rhesus monkeys exposed to applications of H_3AsO_4 in water showed that the skin absorbed from 6.4 +/− 3.9 percent from a low dose of 0.00004 μg/ cm^2 and 2.0 +/− 1.2 percent from a high dose 0.6 μg/ cm^2. *In vitro* studies done on human skin showed that the same conditions resulted in 0.93 +/− 1.1 percent of the low dose of arsenic absorbed after 24 hr (Wester et al. 1993). Bernstam et al. (2002) showed that synthetic skin absorbed 0.25 ppb/hour from 10 ppb arsenic(V) water and 1.3 ppb per hour from 100 ppb water. The arsenic concentration in the skin of rats was increased from 0.6 to 27 ppm after being fed 50 ppm of arsenic trioxide for 21 days. Hamster skin levels increased from 0 to 38 ppm and rabbit skin levels increased from 0 to 2.5 ppm under the same conditions (Peoples 1964).

Wood, Soils and Plants

The use of plant materials for artifacts includes wood, reeds and grasses.

The levels of arsenic and mercury in plants vary according to the level of the toxins in the soil that they are growing in. Tobacco seems to be an exception to this and has higher arsenic concentrations in the leaves than the soil. Chickweed, moss and marine algae are a few of the plants that may concentrate mercury (Shacklette 1970). Soil concentration for arsenic ranges from 0.2 to 40 ppm for untreated soils and can run up to 2,500 ppm in orchards and crop lands that were treated with inorganic arsenicals prior to its ban in 1968. Arsenic generally concentrates more in plant roots and tends to be excluded from the seeds and fruit (Walsh et al. 1977).

Rice plants growing from tube well water containing more than 100 ppb arsenic showed levels of 2.4 ppm in the root, 0.73 ppm in the stem and 0.14 ppm in the rice grains (Das et al. 2004). The soil in the Das study had arsenic concentrations from 7.31 to 27.28 ppm. Total arsenic content for grains (flour and corn) was found to be ~ 40 ppb, while rice and rice products contained 303 ppb (Yost et al. 2004).

A study done on the marine kelp, alaria mana, indicated levels of arsenic at 29.4 ± 1.3 ppm and mercury 0.05 ± 0.01 ppm on a dry weight basis (Burger et al. 2007b).

Table 4. The levels of mercury found in wood.

Arsenic or mercury levels found in wood	Species	Location/ environment
500 ppb Hg	Red Cedar	Missouri/normal soil Conditions
1,000 ppb Hg	Alder	Lower Yukon River District, Alaska / plants growing in soil over cinnabar veins.
1,000–1,500	Black Spruce	
500–1,000	Dwarf birch	
1,000–3,000	Labrador tea	
3,000	Spiraea	
500–2,000	White birch	
Less than 500 ppb Hg	Post Oak	Missouri/normal soil conditions
	Over-up oak	
	Smooth sumac	
	Red Cedar	

Aquatic plants such as reeds grown near geothermal sources of water that contained 1 to 3 ppm of arsenic in Yellowstone National Park, USA, showed levels of arsenic from 118.9 to 258.5 ppm, whereas terrestrial grasses in the same area had levels ranging from 0.4 to 28.5 ppm (Kocar et al. 2004).

A study done on Flemish soils indicates that the median trace concentration of arsenic was 7 ppm while the median mercury concentration was 0.17 ppm (Tack et al. 1997). This study suggested that the clay content and organic carbon content of the soil have a significant impact on the accumulation of toxins, while another later study showed that it did not (Tack et al. 2005). Arsenic mobilization was studied in dolomite and sandstone deposits, and ferric oxyhydroxides were found to retard mobilization (Thornburg and Sabia 2004).

Carey et al. (1980) showed that the mercury and arsenic levels in urban soils was nearly double the levels found in suburban soils. This is thought to be due to the burning of fossil fuels and industrial waste.

The mercury content of rocks in the earth's crust range from 10 to 20,000 ppb. 80 percent of recorded rock samples having less than 1000 ppb. Mercury content in soils averages about 100 ppb. Mercury levels in sedimentary rocks of the Colorado River Plateau range from $<$10 ppb to 10,000 ppb (Cadigan 1971). Soils that support vegetation contain about 10 to 300 ppb mercury. The mercury content of plants and woods studied by Shacklette (1970) is summarized in Table 4. Shacklette showed that the mercury levels varied according to mercury soil content and in cases where roots penetrated into cinnabar deposits, mercury levels ran exceptionally high.

From this data we would expect normal levels of mercury in wood to be at 500 ppb or below. This is a reasonable expectation for arsenic as well, considering the rice stem data as an approximation. The soil and water mercury and arsenic levels in the area that the plant matter came from will cause a large variation in this value, and if the root (versus the branch) of the tree was used in the artifact (as is common for Hopi Kachinas carved from Cottonwood tree roots), the toxin levels will be increased.

Conclusions

Literature indicating the baseline levels of arsenic and mercury in materials commonly used to make artifacts have been reviewed. The natural levels of arsenic

Table 5. Summary of the level of arsenic and mercury found in materials used to make artifacts.

Material	Normal As/Hg mean levels	References
Hair (human)	As 0.04–1.04 ppm Hg 0.06–6.1 ppm	Ali et al. 2003; Foo et al. 1988; dePeyster et al. 1995; Gibson et al. 1982; Oskarsson et al. 1994; Pesch et al. 2002; Saad et al. 1994.
Hair or Fur (animals)	As 20 ppm or less Hg 20 ppm or less	Ben-David et al. 2001; Cumbie et al. 1975; Duffy et al. 2005; Evans et al. 1998; Kocar et al. 2004; Sheffy et al. 1982; Stevens et al. 1997.
Skin	Mammals:	
	As 4 ppm or less Hg no data	Kocar et al. 2004
	Reptiles:	
	As 0.15–167 ppb Hg 90–917 ppb	Burger et al. 2007a; Campbell et al. 2005
Feathers	As 0.05–9.16 ppm Hg 0.01–22.3 ppm	Becker et al. 2002; Burger et al. 2000; Montiero et al. 1998; Palma et al. 2005; Veerle et al. 2004.
Wood and other plants	As less than 1.0 ppm Hg less than 0.5 ppm	Bassey et al. 2007; Burger et al. 2007b; Das et al. 2004; Shacklette et al. 1973.
Soils	As 0.2–40 ppm Hg 0.01–20 ppm	Cadigan et al. 1973; Carey et al. 1980; Shacklette 1970; Tack et al. 1997.

and mercury have been shown to vary depending on soil and water conditions and, in the case of mammals, diet. Table 5 is a summary of the information presented showing typical ranges of toxins found in materials that make up artifacts. These values can be used to help evaluate when an artifact is actually contaminated as well as an estimated end-point for decontamination.

In general, if levels of arsenic or mercury are found that are 1 ppm or above (the lower detection level of a hand-held XRF commonly used to non-destructively test artifacts), the artifact should be suspected of being contaminated with insecticides and levels should be considered only natural after visual inspection and spot testing. Further tests for lower levels of contamination may be warranted to completely rule out lower levels of contamination, depending on the environment and the use the artifact is slated for. This determination takes a team composed of tribal members, a conservator, a chemist and a medical toxicologist.

Literature Cited

Abdel-Rahman, M.S., G.A. Skowronski, and R.M. Turkall. 2005. *In vitro* penetration of pig skin by heavy metals in soil. Soil & Sediment Contamination 14(2):123–134.

Ali, M. and S.A. Tarafdar. 2003. Arsenic in drinking water and in scalp hair by EDXRF: A major recent health hazard in Bangladesh. Journal of Radioanalytical and Nuclear Chemistry 256(2):297–305.

Appelquest, H., S. Asbirk, and I. Drab. 1984. Mercury monitoring: Mercury stability in bird feathers. Marine Pollution Bulletin 15(1):22–24.

Bassey, D.E. and D.U. Essien. 2007. Correlation between levels of some heavy metals in plants and sediments from a segment of Calabar River and associated creeks in Cross River state of Nigeria. Toxicological and Environmental Chemistry 89(1):7–14.

Becker, P.H., J. Gonzalez-Solis, B. Behrends, and J. Croxall. 2002. Feather mercury levels in seabirds at South Georgia: Influence of trophic position, sex and age. Marine Ecology: Progress Series 243:261–269.

Belluck, D.A., S.L. Benjamin, P. Baveye, J. Sampson, and B. Johnson. 2003. Widespread arsenic contamination of soils in residential areas and public spaces: An emerging regulatory or medical crisis? International Journal of Toxicology 22(2):109–128.

Ben-David, M., L.K. Duffy, G.M. Blundell, and R.T. Bowyer. 2001. Natural exposure of coastal river otters to mercury: Relation to age, diet, and survival. Environmental Toxicology and Chemistry / SETAC 20(9):1986–1992.

Bernstam, L., Cheng-HangLan, J. Lee, and O. Nriagu. 2002. Effects of arsenic on human keratinocytes: Morphological, physiological, and precursor incorporation studies. Environmental Research 89(3):220–235.

Burger, J. and M. Gochfeld. 1996. Heavy metal and selenium levels in birds at Agassiz National Wildlife Refuge, Minnesota: Food chain differences. Environmental Monitoring and Assessment 43(3):267–282.

Burger, J. and M. Gochfeld. 1997. Heavy metal and selenium concentrations in feathers of egrets from Bali and Sulawesi, Indonesia. Archives of Environmental Contamination and Toxicology 32(2):217–221.

Burger, J. and M. Gochfeld. 2000. Metal levels in feathers of 12 species of seabirds from midway atoll in the northern Pacific Ocean. Science of the Total Environment 257(1):37–52.

Burger, J., K.R. Campbell, S. Murray, T.S. Campbell, K.F. Gaines, C. Jeitner, T. Shukla, S. Burke, and M. Gochfeld. 2007a. Metal levels in blood, muscle and liver of water snakes (Nerodia spp.) from New Jersey, Tennessee and South Carolina. Science of the Total Environment 373:556–563.

Burger, J., M. Gochfeld, C. Jeitner, M. Gray, T. Shukla, S. Shukla, and S. Burke. 2007b. Kelp as a bioindicator: Does it matter which part of 5M long plant is used for metal analysis? Environmental Monitoring & Assessment 128:311–321.

Cadigan, R.A. 1971. Geochemical distribution of some metals in the Moenkopi formation and related strata of the Colorado Plate. Geological Survey Bulletin 1344, Washington, U.S. Government Printing Office. 56 pp.

Campbell, K.R., T.S. Campbell, and J. Burger. 2005. Heavy metal concentrations in northern water snakes (Nerodia sipedon) from East Fork Poplar Creek and the Little River, East Tennessee, USA. Archives of Environmental Contamination & Toxicology 49:239–248.

Carey, A.E., J.A. Gowne, T.J. Forehand, H. Tai, and G.B. Wiersma. 1980. Soils. Pesticides Monitoring Journal 13(4):150–154.

Carter, D.E., H.V. Aposhian, and A.J. Gandolfi. 2003. The metabolism of inorganic arsenic oxides, gallium arsenids, and arsine: A toxic-chemical review. Toxicology and Applied Pharmacology 193:309–334.

Cumbie, P.M. 1975. Mercury levels in Georgia otter, mink and freshwater fish. Bulletin of Environmental Contamination & Toxicology 14(2):193–196.

Das, H.K., A.K. Mitra, P.K. Sengupta, A. Hossain, F. Islam, and G.H. Rabbani. 2004. Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environment International 30(3):383–387.

dePeyster, A. and J.A. Silvers. 1995. Arsenic levels in hair of workers in a semiconductor fabrication facility. American Industrial Hygiene Association Journal 56(4):377–383.

Duffy, LawrenceK., R.S. Duffy, G. Finstad, and C. Gerlach. 2005. A note on mercury levels in the hair of Alaskan reindeer. Science of the Total Environment 339(1–3):273–276.

Dutiewicz, T. 1977. Experimental studies on arsenic absorption routes in rats. Environmental Health Perspectives 19:173–177.

Evans, R.D., E.M. Addison, J.Y. Villeneuve, K.S. MacDonald, and D.G. Joachim. 1998. An examination of the spatial variation in mercury concentrations in otter (Lutra canadensis) in south-central Ontario. Science of Total Environment 213:239–245.

Feng, Q., Y. Suzuki, and A. Hisashige. 1998. Hair mercury levels of residents in China, Indonesia, and Japan. Archives of Environmental Health 53(1):36–43.

Focazio, M.J., A.H. Welch, S.A. Watkins, D.R. Helsel, and M.A. Horn. 2000. A retrospective analysis on the occurrence of arsenic in ground-water resources of the United States and limitations in drinking-water-supply characterizations: U.S. Geological Survey Water- Resources Investigation Report 99-4279. 27 pp.

Foo, S.C., C.H. Ngim, W.O. Phoon, and J. Lee. 1988. Mercury in scalp hair of healthy Singapore residents. Science of the Total Environment 72:113–122.

Foust, R.D. Jr., P. Mohapatra, A.M. Compton-O'Brien, and J. Reifel. 2004. Groundwater arsenic in the Verde Valley in central Arizona, USA. Applied Geochemistry 19:251–255.

Gebel, T., S. Kevekordes, J. Schaefer, H. von Platen, and H. Dunkelberg. 1996. Assessment of a possible genotoxic environmental risk in sheep bred on grounds with strongly elevated contents of mercury, arsenic and antimony. Mutation Research 368(3–4):267–274.

Gibson, R.S. and L.A. Gage. 1982. Changes in hair arsenic levels in breast- and bottle-fed infants during the first year of infancy. Science of the Total Environment 26(1):33–40.

Goeda, A.A. and M. de Bruin. 1984. The use of bird feather parts as a monitor for metal pollution. Environmental Pollution (Series B) 8:281–298.

Hostynek, J.J., R.S. Hinz, C.R. Lorence, M. Price, and R.H. Guy. 1993. Metals and the skin. Critical Reviews in Toxicology 23(2):171–235.

Kocar, B.D., R.A. Garrott, and W.P. Inskeep. 2004. Elk exposure to arsenic in geothermal watersheds of Yellowstone National Park, USA. Environmental Toxicology and Chemistry 23(4):982–989.

Kwon, E., H. Zhang, Z. Wang, G.S. Jhangri, X. Lu, N. Fok, S. Gabos, Xing-FangLi, and X.C. Le. 2004. Arsenic on the hands of children after playing in playgrounds. Environmental Health Perspectives 112(14): 1375–1380.

Martin, G. and M. Kite. 2003. Conservator safety—Mercury in felt hats. Pp. 177–181 in *Proceedings of Conservation Science 2002 Conference, Edinburgh May 22–24* (J. Townsend, K. Eremin, and A. Adriaens, eds.). Archetype Publications, London.

Monteiro, L.R., J.P. Granadeiro, and R.W. Furness. 1998. Relationship between mercury levels and diet in Azores seabirds. Marine Ecology: Progress Series 166:259–265.

Ndiokwere, C.L. 1985. A survey of arsenic levels in human hair and nails—Exposure of wood treatment factory employees in Nigeria. Environmental Pollution, Series B: Chemical and Physical 9(2):95–105.

Odegaard, N. and A. Sadongei. 2001. The issue of pesticides on Native American cultural objects: A report on conservation and education activities at the University of Arizona. Collection Forum 16(1–2): 12–18.

Oskarsson, A., B.J. Lagerkvist, B. Ohlin, and K. Lundberg. 1994. Mercury levels in the hair of pregnant women in a polluted area in Sweden. Science of the Total Environment 151(1):29–35.

Palma, L., P. Beja, P.C. Tavares, and L.R. Monteiro. 2005. Spatial variation of mercury levels in nesting Bonelli's eagles from Southwest Portugal: Effects of diet composition and prey contamination. Environmental Pollution (Amsterdam, Netherlands.) 134(3):549–557.

Palmer, P.T. 2001. A review of analytical methods for the determination of mercury, arsenic, and pesticide residues on museum objects. Collection Forum 16(1–2):25–41.

Pecore, W.T. 1970. Mercury in the Environment, Geological Survey Professional Paper. 1–5.

Peoples, S.A. 1964. Arsenic toxicity in cattle. Annals of the New York Academy of Sciences 111(2):644–649.

Pesch, A., M. Wilhelm, U. Rostek, N. Schmitz, M. Weishoff-Houben, U. Ranft, and H. Idel. 2002. Mercury concentrations in urine, scalp hair, and saliva in children from Germany. Journal of Exposure Analysis and Environmental Epidemiology 12(4):252–258.

Saad, A. and M.A. Hassanien. 2001. Assessment of arsenic level in the hair of the non-occupational Egyptian population: Pilot study. Environment International 27(6):471–478.

Seidel, A., R. Kreutzer, D. Smith, S. McNeel, and D. Gilliss. 2001. Assessment of commercial laboratories performing hair mineral analysis. JAMA 285(1):67–72.

Shacklette, H.T. 1970. Mercury content of plants. Pp. 35–36 in *Mercury in the Environment. US Geological Survey Professional Paper No. 713*. United States Government Printing Office, Washington DC. 67 pp.

Shacklette, H.T. and J.J. Connor. 1973. Airborne chemical elements in Spanish moss. Geological Survey Professional Paper 574-E, Washington, U.S. Government Printing Office. 56 pp.

Sheffy, T.B. and J.R. St. Amat. 1982. Mercury burdens in furbearers in Wisconsin. Journal of Wildlife Management 46(4):1117–1120.

Sirois, P.J. 2001. The analysis of museum objects for the presence of arsenic and mercury: Non-destructive analysis and samples analysis. Collection Forum 16(1–2):65–75.

Stevens, R.T., T.L. Ashwood, and J.M. Sleeman. 1997. Mercury in hair of muskrats (Ondatra zibethicus) and mink (Mustela vison) from the U.S. Department of Energy Oak Ridge Reservation. Bulletin of Environmental Contamination & Toxicology 58:720–725.

Swain, E.B., D.R. Engstrom, M.E. Brigham, T.A. Henning, and P.L. Brezonik. 1992. Increasing rates of atmospheric mercury deposition in midcontinental North America. Science 257:784–787.

Tack, F.M.G., M.G. Verloo, L. Vanmechelen, and E. Van Ranst. 1997. Baseline concentration levels of trace elements as a function of clay and organic carbon contents in soils in Flanders (Belgium). Science of the Total Environment 201(2):113–123.

Tack, F.M.G., T. Vanhaesebroeck, M.G. Verloo, K. Van Rompaey, and E. Van Ranst. 2005. Mercury baseline levels in Flemish soil (Belgium). Environmental Pollution 134:173–179.

Thornburg, K. and N. Sahia. 2004. Arsenic occurrence, mobility and retardation in sandstone and dolomite formations of the Fox River Valley, Eastern Wisconsin. Environmental Science & Technology 381:5087–5094.

Veerle, J., D. Tom, P. Rianne, B. Lieven, B. Ronny, and E. Marcel. 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, Parus major. Journal of Environmental Monitoring 6(4):356–360.

Walsh, L.M., M.E. Sumner, and D.R. Keeney. 1977. Occurance and distribution of arsenic in soils and plants. Environmental Health Perspectives 19:67–71.

Wester, R.C., H.I. Maibach, L. Sedik, J. Melendres, and M. Wade. 1993. *In vivo* and *in vitro* percutaneous absorption and skin decontamination of arsenic from water and soil. Fundamental and Applied Toxicology 20(3):336–40.

Wester, R.C., H. Xiaoying, S. Barbadillo, H.I. Maibach, Y.W. Lowney, R.A. Schoof, S.E. Holm, and M.V. Ruby. 2004. *In vivo* percutaneous absorption of arsenic from water and CCA-treated wood residue. Toxicological Sciences: An Official Journal of the Society of Toxicology 79(2):287–295.

Yost, L.J., S.H. Tao, S.K. Egan, L.M. Barraj, K.M. Smith, J.S. Tsuji, Y.W. Lowney, R.A. Schoof, and N.J. Rachman. 2004. Estimation of dietary intake of inorganic arsenic in U.S. children. Human and Ecological Risk Assessment 10(3):473–483.

THE EFFECT OF PH ON ETHANOL PRESERVED MUSCLE TISSUE

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Abstract.—Maintaining stable chemical conditions in fluid preserved collections is desirable for long term specimen conservation. A key issue that can cause degradation in such collections is the pH of the preservation fluid, since extremes of pH promote damaging chemical reactions. This study looked at pH changes caused by muscle tissue samples preserved in 80% ethanol solutions, conditioned to a range of different pH levels, and monitored for a period of 240 days. The samples were then analysed using FTIR (Fourier Transformed Infrared) spectrometry to assess any possible chemical changes in the preserved muscle tissue from the differing pH environments. The pH across the samples settled into the range of pH 5.3 to 7.2, while multivariate analysis of the FTIR spectra suggested chemical differences between the samples initially preserved at pH 3–5 to those preserved at pH 7–13. The study also highlighted the problems with using pH as a measure due to the way the pH scale behaves in non aqueous solvents such as ethanol and due to the difficulties reliably measuring pH. Despite this, it is felt this study adds to our knowledge on the behaviour of preserved tissues in ethanol solutions.

INTRODUCTION

Many factors affect the long term preservation of fluid preserved tissues such as the type of organism being preserved, the fixative and/or preservation chemicals used, and the storage environment (Horie 1989, Cato 1990, Simmons 1995, 2002). The importance of correct fixation and preservation solutions with fluid preserved museum collections has been extensively discussed (e.g., Steedman 1976, Dingerkus 1982, Stoddard 1989, Simmons 2002). In addition some researchers have been considering the effects of these methodologies on specific biomolecules such as DNA (e.g., Post et al. 1993, Austen and Dillon 1997, Vink et al. 2005, Zimmermann et al. 2008). The issue of pH levels in preserving solutions has long been recognised as an important factor (Steedman 1976, Dingerkus 1982, Stoddard 1989, Simmons 2002, Hargrave et al. 2005). Acidic conditions can decalcify bone and promote other chemical changes, while alkaline conditions can gelatinise and denature proteins. Thus, understanding the effects of pH within the fluid preservation environment a specimen is stored in is desirable to improve the long term conservation and value of archival fluid preserved collections.

Simmons (1995, 2002) provides valuable overviews of the issues affecting fluid collections. A number of specific studies have considered the effects of pH within fluid preserved collections, although these have tended to focus on the acidity problems within formaldehyde solutions (Steedman 1976, Stephensen and Riley 1994, Carter 1997). However studies such as Cato (1990), Andrei and Genoways (1999) and Hargrave et al. (2005) have looked at ethanol preserved collections, while Waller and Strang (1996) have extensively reviewed the physical properties of ethanol. Hargrave et al. (2005) highlighted that the pH is affected by a range of interacting factors that can affect the long term preservation of a specimen. In addition, accurately measuring the pH of mixed solutions such as diluted alcohol is difficult to achieve (Frant 1995), an issue discussed by Waller et al. (1993) and encountered by Waller and Simmons (2003) in their comprehensive assessment of a fluid preserved herpetological collection. The pH scale differs in water-miscible non-aqueous solvents and large measurement errors are possible. This makes pH results from different studies difficult to directly compare. Nonetheless, if the solvent

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background remains constant then pH measurements are reasonably reproducible and usable within studies looking at acidity changes as this provides useful information on the changes in hydrogen ion concentrations.

With this in mind, this small scale study was carried out with the aim of following the behaviour of the pH in a single type of preservation environment. Additional characteristics such as type of labels and variable storage environments were not explored. Whilst this limits the scope of this study, it is considered that the results obtained add to our knowledge on the way fluid preserved collections potentially behave. A series of preserved tissue samples were prepared and preserved in an 80% ethanol solution which was conditioned to different pH levels. The pH was then monitored for a period of time before a final analysis of the tissue samples using Fourier Transformed Infra Red (FTIR) spectroscopy. Spectral data was analysed to assess possible differences in protein structure in the muscle tissue samples across the different pH treatments. This involved using the spectral processing function of de-convolution (Stuart 1997) to improve spectral resolution and explore steric changes in the protein chemistry using the Amide I and Amide II peaks. The Amide I peak is particularly useful in protein studies as it is sensitive to small variations in molecular geometry and hydrogen bonding patterns within proteins. The band is due mainly to vibrational changes in the carbonyl bond, C = O, of the peptide linkages that constitute the backbone structure of the proteins (Dong et al. 1995, Barth 2007). Spectral differences were further analysed using the multivariate statistical method of Principle Components Analysis (PCA) to explore and visualise variance in the spectral data, based on the techniques utilised by Malins et al. (1997).

Materials and Methods

All pH measurements were taken using a Jenway double junction glass combination electrode, specified for use with ethanol solutions, on a Whatman PHA230 meter. The meter was calibrated prior to taking pH readings of the study solutions and all readings were allowed to stabilise before taking the pH reading to the nearest 0.1 unit. The electrode was rinsed in de-ionised water between each reading. Once pH readings had been taken the pH meter was recalibrated and the readings repeated to check for reproducibility.

A stock preserving solution of 80% ethanol was prepared using 0.5 M sodium acetate in deionised water (pH 8.5). The sodium acetate was added as a buffer salt to help maintain the chosen pH of the solution and was used in all of the conditioned solutions to ensure the tissue samples were treated as equally as possible. Aliquots of this solution were then conditioned to a range of pH strengths (pH 3.0, pH 5.0, pH 7.0, pH 9.0, pH 13.0) using 1 M hydrochloric acid or 0.5 M sodium hydroxide as appropriate. At the extremes of pH this did create an additional dilution effect reducing the overall ethanol concentration but as this was limited to a few percent it was ignored in this study. A control preserving solution was also prepared using 80% ethanol in deionised water. Four samples from each pH level were then prepared by taking 15 ml aliquots and placing them in 20 ml Wheaton glass scintillation tubes. After 24 hr the pH was again measured before adding equal sized pieces of pork tissue of approximately 1 cm^3 total volume into three of the four prepared scintillation tubes for each pH level. The fourth tube acts as a control to monitor the pH behaviour of the preserving solution without the addition of the tissue sample. Samples were prepared using the control preserving solution in the same way. The pH of the samples was then monitored at various intervals over a period of 240 days.

At the end of the pH monitoring period the tissue samples were then analysed for possible differences in their chemistry using FTIR spectroscopy using a Perkin Elmer Spectrum One spectrometer. Small samples of muscle tissue were removed using a clean scalpel blade and then briefly washed in deionised water. The muscle tissue was then blotted dry before analysis using the Universal ATR (attenuated total reflection) accessory. Each spectrum was collated from 10 scans at a resolution of 4 cm^{-1}. A minimum of three separate spectra were collected from each tissue sample using the Spectrum 5.0 software. After collection each spectrum was further processed. The first process used deconvolution to enhance spectral data by resolving overlapping bands (Stuart 1997). The second process normalised the original data on the main Amide I peak characteristic of the protein component (see Fig. 2). The spectral data was then saved as an ASCII file and exported to Excel where all spectral wave numbers outside the main protein fingerprint region of 1,800–1,400 cm^{-1} were removed. This spectral data was further analysed using the multivariate method of PCA with the statistical analysis programme PAST (Hammer et al. 2001) and the data visualised on a scatter plot.

Results

Figure 1a and b show the changes in pH over the period of the study for both the control solutions and the sample containing solutions respectively. All of the control samples held a steady pH at, or close to, the start value throughout the study apart from the overall control sample which contained no sodium acetate buffer salt (labelled 'No Buffer'). This had an initial pH drop from pH 9.5 to 8.5 but then remained steady at pH 8.0 for the rest of the study. However when the tissue samples were added to the preserving solutions there were notable changes in the pH level, especially with the solutions conditioned to the extreme pH levels. Within a 20 day period all the samples had settled into the region of pH 5.3–7.2, remaining steady for the course of the study, as shown in Figure 1b. Statistical comparison was not carried out due to the small size of the sample sets. However there was little variation in the pH readings from each triplicate set of samples (+/−0.2 pH units was the greatest variation recorded) and the readings proved reproducible with the repeated measurements.

Figure 2 shows a typical FTIR spectrum obtained in the study which is clearly dominated by the Amide I and Amide II bands typical of protein chemistry. This was explored further by looking directly at the spectral data. Examples are shown in Figure 3 with detail from the Amide I and Amide II bands using the de-convolved spectral data from tissue samples initially preserved at pH 3.0; pH 7.0 and pH 13.0. While small differences in the spectra are present these are not obviously different between the samples from the different pH environments, and thus no differences in chemical structure could be resolved from this information. However Figure 4a illustrates the PCA analysis of this region of the spectrum from 1,800–1,400 cm^{-1} wave-numbers with the data from all tissue samples. Each sample set has been delimited by convex hulls. Within this there a separation between the pH 3–5 samples, and the pH 7–13 samples, with the control tissue samples crossing the two groupings. In Figure 4b the control tissue samples have been removed and the samples grouped into the pH 3–5 and the pH 7–13 sample sets. The separation of the two groups suggests a difference in the chemical effects on the muscle tissue between the two groupings. Thus the PCA analysis indicates there are pH induced changes with the chemical structure of the preserved muscle tissue proteins.

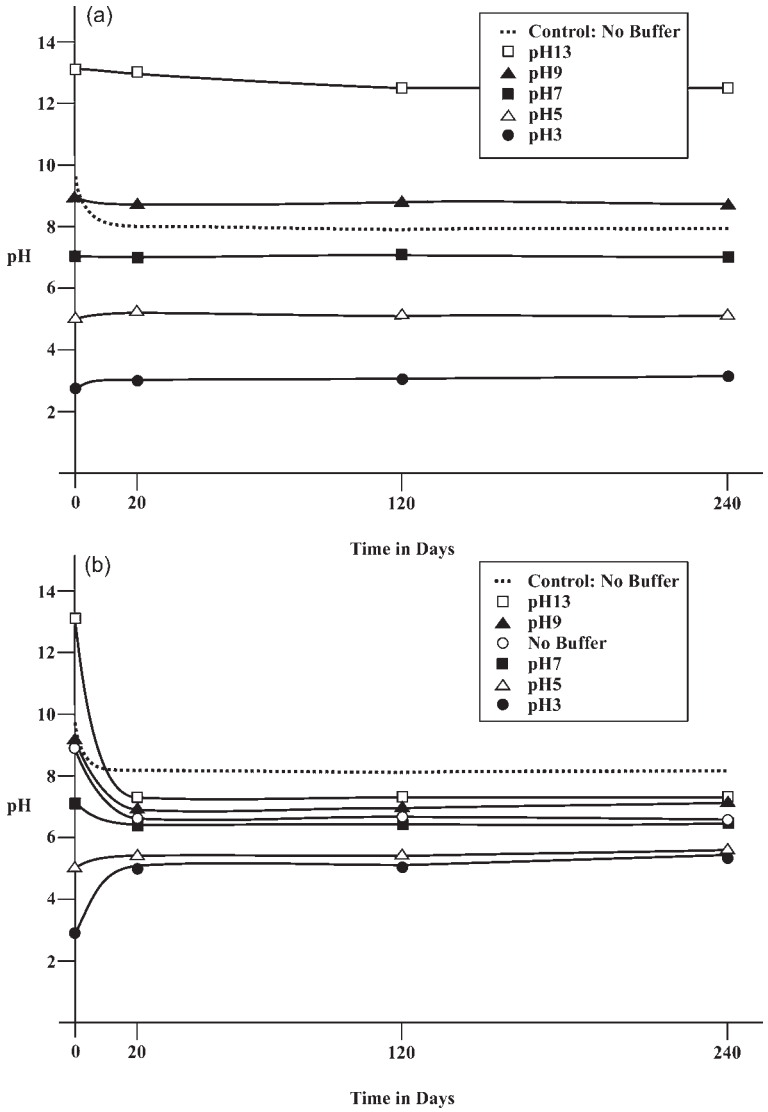

Figure 1. Changes in pH over the course of the study period: (a) shows the pH in the ethanol solutions without the addition of the tissue samples. (b) Shows the pH changes in the ethanol solutions containing tissue samples. The 'control: no buffer' is the same in each graph.

Discussion

It is interesting to note that the addition of the specimen samples to the preserving fluids caused the pH levels in all the samples to alter to broadly similar values within the first 20 days of the study, and this is despite the presence of the sodium acetate salt in the test solutions, particularly with the low pH samples. This is a similar pattern to that observed in the results in Hargrave et al. (2005) where the pH settled at around pH 7.0 with ethanol samples in distilled water, within the study by Cato (1990), and is also broadly consistent with the data recorded for ethanol preserved specimens in Waller and Simmons (2003), but this must be considered with the comments in the introduction to

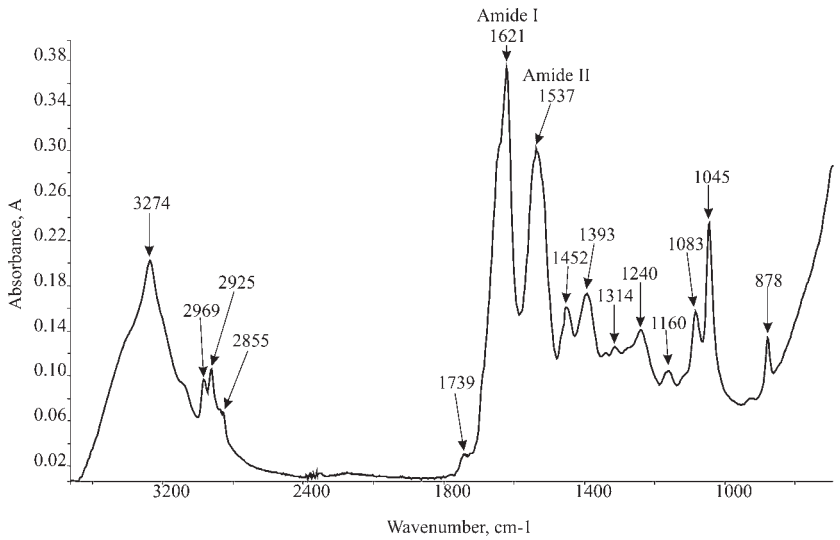

Figure 2. Typical FTIR spectrum obtained in this study showing the characteristic Amide I and Amide II peaks.

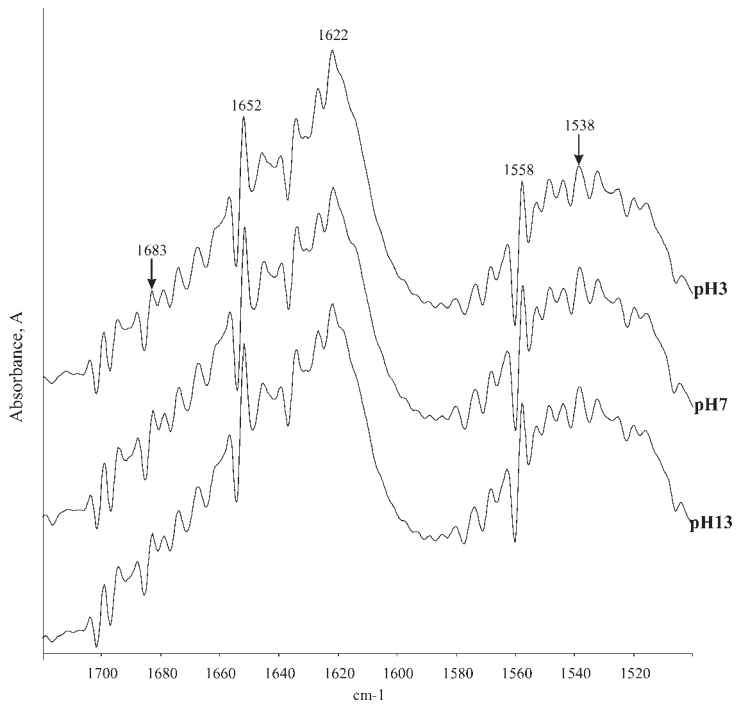

Figure 3. Deconvolved spectra for the pH 3, 7 and 13 tissue samples in the region of the Amide I and Amide II peaks.

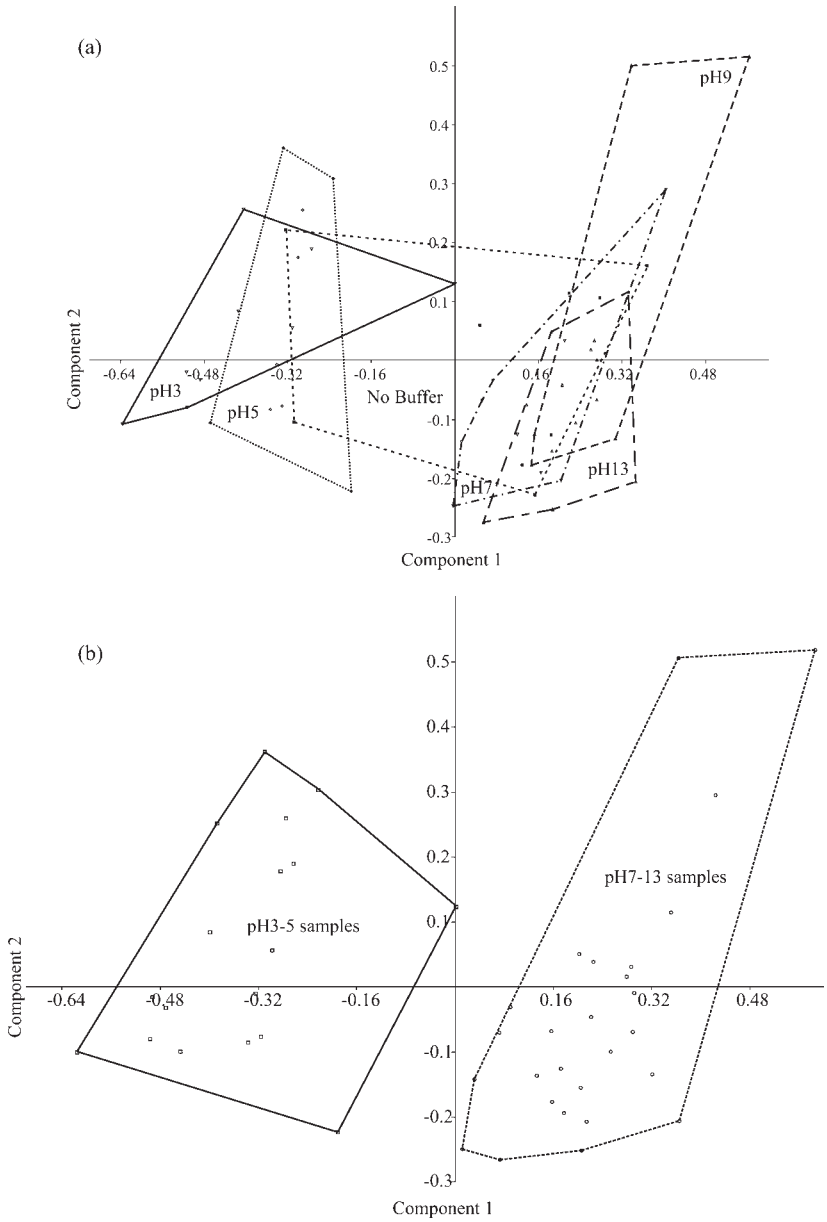

Figure 4. Scatterplots of the PCA analysis of tissue samples delimited by convex hulls. (a) All sample groups. PC1 = 0.0900302, PC2 = 0.0347977. (b) Samples divided into two groups; pH 3–5 and pH 7–13, with the control samples removed. PC1 = 0.0962656, PC2 = 0.0373132.

this paper stating that readings between studies are not directly comparable (Frant 1995) and which is discussed further below.

The question of the effects of acidity in ethanol solutions used to preserve animal specimens is a complex issue and is made more difficult by the problems of accurately monitoring the pH in such solutions (Waller et al. 1993, Frant 1995), and by the confusion that exists with using pH (Bates and Popovych 1981), though in most cases the

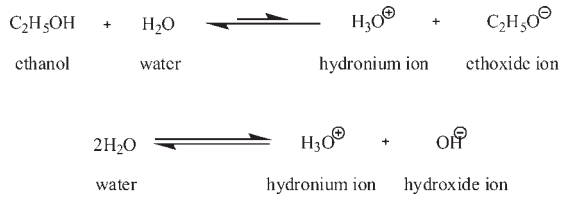

Figure 5. The upper equation shows the loss of a proton on the alcohol group of ethanol to form the ethoxide ion, although in an aqueous solution water is more acid and hydroxide ion formation is preferred as shown in the lower equation.

definition is being used as in the Bronsted-Lowry theory (Dickerson et al. 1979). Overall pH is a measure of the acidity or alkalinity of a solution. It is defined as the co-logarithm of the activity of dissolved hydrogen ions (H+). Hydrogen ion activity coefficients cannot be measured experimentally, so they are based on theoretical calculations. Also the pH scale is not an absolute scale; it is relative to a set of standard solutions whose pH is established by international agreement (IUPAC 2001). This becomes more complex again when dealing with mixed and non-aqueous solutions (Frant 1995) and highlights the difficulty in measuring pH in high concentration ethanol solutions such as those used in this study. Interestingly Frant (1995), using the dielectric constant (e) for ethanol, states the neutral pH (where there are equal amounts of $C_2H_50^-$ and H^+) for ethanol as 9.55 using the water based scale, and that the scale now goes from -4.2 to 14.9 units. However most text books state a range from pH 7.3 to 7.9 based on a pKa (acid dissociation constant) of 15.9. This reinforces the confusion with pH and introduces the question of whether the level of desirable pH such as between 6.0 and 7.0 for the preservation of collections is in fact meaningful with ethanol preservation. This figure is generally quoted as it is near the isoelectric point (pI) for many key proteins, which is the point at which the protein is carrying no net charge and is at its least soluble, and is based on how proteins behave in a highly aqueous environment and not in that of an ethanol solution in which the pH scale is behaving differently. Ethanol itself can have the tendency to lose the proton on the alcohol group to form the ethoxide ion (Fig. 5). However in an aqueous solution water is more acid and hydroxide ion formation is preferred (Morrison and Boyd 1972). Thus with partly diluted ethanol solution, as used in this study, it may be that the ethoxide ion may also form due to the lower concentrations of water, but this requires an understanding of the chemical equilibria as discussed by Waller et al. (1993). With such solutions then there is a difference in the acid–base equilibrium constants compared to a highly aqueous solution. To interpret acid-base equilibrium pairs based on pH measurements from solutions of different ethanol concentrations it is necessary to know the dielectric constant (e). This is a measure of the ability of a dielectric material to store electrical potential energy under the influence of an electric field. Miscible organic solvents such as ethanol decrease the dielectric constant of water, which in effect allows proteins to come closer together resulting in an altering of their solubility. This is undoubtedly complicated further by the whole solvent environment and chemical interactions with the biological tissue being preserved. Another way to measure acidity in ethanol based solutions could be the approach used by the bio-ethanol industry which measures the acid strength, as measured by pHe, as this is a good predictor of the corrosion potential of ethanol fuels. It is preferable to the pH or total acidity because this does not measure acid strength; overestimates the contribution of weak acids, such as carbonic acid; and may underestimate the corrosion potential of low concentrations of

strong acids. Acid strength is determined by measuring the pHe of fuel, which is similar but not directly comparable to the pH of a water solution. pHe is defined as "a measure of the acid strength of alcohol fuels defined by this apparatus and procedure." (ASTM 2008).

Only a limited number of detailed studies have looked at the chemical changes occurring in fluid preserved collections. Some detailed studies do exist such as the valuable studies of Von Endt (1994 and 2000) using gas chromatography and mass spectrometry techniques, and Genter and Wentrup-Byrne (1999) which used FTIR to look at preservation induced changes on nematodes. This study also used FTIR analysis in an attempt to determine chemical changes, and the processes used gave mixed results. While the detailed analysis of the Amide I and II bands using deconvolution did not show any clear differences in the overall structure of the proteins the use of multivariate techniques did. Spectral processing by deconvolution is a useful tool but more specific work is required to explore possible changes in protein structure through this methodology. The PCA analysis is a valuable method for analysing differences in similar looking data sets, and its use with the FTIR data is indicating changes in the chemical structure and/or conformation of the preserved muscle tissue from the differing pH environments. This is what is expected as the pH affects the charge on the protein molecules (Creighton 1984, Casal et al. 1988, Pace et al. 2004) altering the conformation of the protein. The whole issue of protein stability is very complex as the native structure of proteins are only marginally stable under the best of conditions and can be affected by many factors such as temperature or the presence of denaturants as well the pH (Creighton 1984). Proteins hold their structure in a number of key ways, the most important of which are hydrophobic interactions, hydrogen bonding and polar group burial (Pace et al. 2004). In ethanol, proteins are generally considered to unfold, and then refold into rod-like structures with a high content of alpha helices (Hirota-Nakaoka and Goto 1999). This buries the polar peptide groups, contributing to the stability of the now denatured protein. As mentioned previously, proteins at their isoelectric point show their minimal solubility, and this lowers further when they are denatured. When a protein becomes charged its solubility increases, although a larger charge is required in the denatured state than the native state to solubilise the protein (Pace et al. 2004) as can occur with changes in the pH altering the ionized state of the protein. However it must be remembered that the changes shown in this study are on broad muscle tissue chemistry and not on purified proteins as used in more specific studies (e.g., Casal et al. 1988, Blanch et al. 1999). This makes defining specific structural changes in protein chemistry harder to define. In addition other key chemical components found in preserved tissues such as fatty acids and lipids have not been considered. Nevertheless it is considered these results offer some further useful insight into the behaviour of ethanol preserved animal tissues.

Conclusions

This study highlighted a number of issues such as the fact that the pH scale behaves differently in water miscible non-aqueous solvents. Any study using pH as a measurement needs to be aware of the limitations in the results obtained, and that it is essential to use good quality equipment such as a specified double junction glass electrode. Further, recommendations for pH levels in fluid preserved collections are based on the behaviour of key chemical groups such as proteins behaviour in an aqueous based fluid rather than a high concentration ethanol solutions as commonly used to preserve natural science

material due to the pH scale behaving differently in different solvents and the possible effect on the isoelectric points of key proteins. The FTIR analysis results indicate that chemical changes as a result of the pH of the preservation environment do occur, and future studies plan to look at this in further detail.

Literature Cited

Andrei, M.A. and H.H. Genoways. 1999. Changes in pH in museum storage fluids, I—Effects of Resistall paper labels. Collection Forum 13(2):63–75.

ASTM Standard D6423. 2008. *Standard test method for determination of pHe of ethanol, denatured fuel ethanol, and fuel ethanol (Ed75–Ed85)*. ASTM International, West Conshohocken, PA. DOI:10.1520/D6423-08, www.astm.org.

Austin, A.D. and N. Dillon. 1997. Extraction and PCR of DNA from parasitoid wasps that have been chemically dried. Australian Journal of Entomology 36:241–244.

Barth, A. 2007. Infrared spectroscopy of proteins. Biochimica et Biophysica Acta 1767:1073–1101.

Bates, R.G. and O. Popovych. 1981. The modern meaning of pH. Critical Reviews in Analytical Chemistry 10:247–278.

Blanch, E.W., L. Hecht, and L.D. Barron. 1999. New insight into the pH-dependent conformational changes in bovine B-lactoglobulin from Raman optical activity. Protein Science 8:1362–1367.

Carter, J. 1997. An investigation of pH changes in a selection of formaldehyde buffering agents used on a fish parasitology research collection. Collection Forum 13(1):1–10.

Casal, L.H., U. Köhler, and H.H. Mantsch. 1988. Structural and conformational changes of B-lactoglobulin B: An infrared spectroscopic study of the effect of pH and temperature. Biochimica et Biophysica Acta 957:11–20.

Cato, P.S. 1990. Characteristics of a collection of fluid preserved mammals and implications for collection management. Collection Forum 6:53–64.

Creighton, T.E. 1984. *Proteins: Structures and Molecular Principles*. W.H. Freeman and Company, New York.

Dickerson, R.E., H.B. Gray, and G.P. Haight. 1979. *Chemical Principles,* 3rd ed. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA. http://resolver.caltech.edu/CaltechBOOK:1979.001

Dingerkus, A. 1982. Preliminary observations on acidification of alcohol in museum specimen jars. Curation Newsletter, American Society of Ichthyologists and Herpetologists 5:1–3.

Dong, A., S.J. Prestrelski, S.D. Allison, and J.F. Carpenter. 1995. Infrared spectroscopic studies of lyophilisation- and temperature-induced protein aggregation. Journal of Pharmaceutical Sciences 85:415–424.

Frant, M.S. 1995. How to measure pH in mixed and non-aqueous solutions. Todays Chemist at Work June 1995:39–42.

Gentner, J.M. and E. Wentrup-Byrne. 1999. Natural history specimen analysis using micro FTIR attenuated total reflectance spectroscopy and transmission electron microscopy. Spectrochimica Acta Part A 55:2281–2188.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1):9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hargrave, C.W., K.G. Sutherland, E.M. Marsh-Matthews, and W.J. Matthews. 2005. Multiple interacting factors affect pH in museum storage solutions. Collection Forum 19:23–31.

Hirota-Nakaoka, N. and Y. Goto. 1999. Alcohol-induced denaturation of beta-lactoglobulin: a close correlation to the alcohol-induced alpha helix formation of melittin. Bioorganic and Medicinal Chemistry 7:67–73.

Horie, C.V. (ed.). 1989. *Conservation of Natural History Specimens: Spirit Collections*. The Manchester Museum and Department of Environmental Biology, The University of Manchester.

IUPAC. 2001. *The measurement of pH - definition, standards and procedures*. Report of the Working Party on pH, IUPAC Provisional Recommendation. http://www.iupac.org/reports/provisional/abstract01/rondinini_prs.pdf.

Malins, D.C., N.L. Polissar, and S.J. Gunselman. 1997. Infrared spectral models demonstrate that exposure to environmental chemicals leads to new forms of DNA. Proceedings of the National Academy of Sciences, USA 94:3611–3615.

Morrison, R.T. and R.N. Boyd. 1972. *Organic Chemistry,* 2rd ed. Allyn and Bacon, Inc.

Pace, C.N., S. Treviño, E. Prabhakaran, and J.M. Scholtz. 2004. Protein structure, stability and solubility in water and other solvents. Philosophical Transactions of the Royal Society London Series B 359:1225–1235.

Post, R.J., P.K. Flook, and A.L. Millest. 1993. Methods for the preservation of insects for DNA Studies. Biochemical Systematics and Ecology 21(1):85–92.

Simmons, J.E. 1995. Storage of fluid preserved collections. Pp. 161–186 in *Storage of Natural History Collections: A Preventive Conservation Approach* (C.L. Rose, C.A. Hawks, and H.H. Genoways, eds.). Society for the Preservation of Natural History Collections, Iowa City, Iowa. x 1 448 pp.

Simmons, J.E. 2002. *Herpetological Collecting and Collections Management*. Herpetological Circular, Society for the Study of Amphibians and Reptiles, vi 1 153 pp.

Steedman, H.E. 1976. General and applied data on formaldehyde fixation and preservation of marine zooplankton. Pp. 103–154 in *Zooplankton Fixation and Preservation* (H.F. Steedman, ed.). The UNESCO Press, Paris, France. 350 pp.

Stephenson, A.B. and J.L. Riley. 1995. Fixation and preservation of museum marine collections using formaldehyde/glutaraldehyde mixes. Collection Forum 11(2):58–68.

Stoddard, R.W. 1989. Fixatives and preservatives, their effects on tissue. Pp. 1–32 in *Conservation of Natural History Specimens: Spirit Collections* (C.V. Horie, ed.). The University of Manchester, Manchester, England. viii 1 115 pp.

Stuart, B. 1997. *Biological applications of infrared spectroscopy*. Analytical Chemistry by Open Learning (Series). 191 pp.

Vink, C.J., S.M. Thomas, P. Paquin, C.Y. Hayashi, and M. Hedin. 2005. The effects of preservatives and temperatures on arachnid DNA. Invertebrate Systematics 19:99–104.

Von Endt, D.W. 1994. Spirit collections: A preliminary analysis of some organic materials found in the storage fluids of mammals. Collection Forum 10(1):10–19.

Von Endt, D.W. 2000. Staying in shape: A comparative study of collagen and keratin stability in museum storage fluids. Society for the Preservation of Natural History Collections: Program and Abstracts of the 15th Annual Meeting, July 8–14, 2000, Halifax, Nova Scotia. P. 48. [Abstract]

Waller, R., R. Stairs, and B. Njie. 1993. pH measurements in ethanol-water solutions. *Program and Abstracts*. Society for the Preservation of Natural History Collections 8th Annual Meeting. Victoria, B.C. 47 pp.

Waller, R. and J.E. Simmons. 2003. An exploratory assessment of the state of a fluid-preserved herpetological collection. Collection Forum 18:1–37.

Waller, R. and T.J.K. Strang. 1996. Physical chemical properties of preservative solutions. 1. Ethanol – water solutions. Collection Forum 12(2):70–85.

Zimmermann, J., M. Hajibabaei, D. Blackburn, J. Hanken, E. Cantin, J. Posfai, and T. Evans. 2008. DNA damage in preserved specimens and tissue samples: a molecular assessment. Frontiers in Zoology 5:1–18 http://www.frontiersinzoology.com/content/5/1/18

BOOK REVIEW

CARE AND CONSERVATION OF NATURAL HISTORY COLLECTIONS, 1999, David Carter and Annette K. Walker. (Butterworth-Heinemann, Oxford, Great Britain, 226 pp.) There is a real need for specific publications in the field of conservation of natural history collections, certainly in Latin America, where I work as a conservator, and this will be my viewpoint for this review.

Natural history collections are probably the most neglected specialty in conservation in comparison with other areas like art objects, paper documents, ceramics, etc. So in this sense to have a book which gives a compilation of guidelines for the wide range of objects that compose these kinds of collections is always welcome. In Latin America, the priorities and budgets regarding conservation in general and natural history collections in particular, are difficult realities to deal with. What we as conservators try to do, in general, is to work with the most updated information and follow the ''directives'' in a ''theoretical way,'' trying to adapt them to our situation.

This book is a very useful tool for museum staff (especially for collection managers and conservators) because one can find answers and solutions to everyday questions in just one volume. In our countries, the discipline of conservation is still in a developmental stage, so it is very difficult to convince the authorities about the necessity of having of having a decent library in addition to education and training. Usually the responsible party for collections follows the past practices of others (previous staff, other collection managers or conservators, etc.) without knowing how appropriate those approaches really are.

Since our basis for past actions was often flawed, having quality information for each kind of collection is very much appreciated. This book is a reference that can be consulted by subject area. Each chapter covers common significant aspects for each kind of collection in a thorough and organized way.

Some information, like general recommendations about storage, handling, fungal attack, etc. is repeated in many chapters, as if it was meant to be read separately. It seems like the book is a summary of publications from different authors and that makes the reading to be a succession of concepts instead a progression of them. For users, it would be more effective if the book were more of a ''manual.'' That could be achieved by placing some chapters like ''collection environment'' and ''pest management, prevention and control'' at the beginning, instead of at the end, and bringing together some information, already given separately in various chapters, such as adhesives, labeling, storage systems, handling recommendations, etc. Each specific chapter (mammals, plants, entomology, etc.) is very useful for the different specialties, but it becomes difficult to have a general approach when the information is so fragmented.

At major museums there may be a specialist for each collection, but usually, in smaller museums there is one person in charge of all kinds of natural history materials. In this sense, I think that a more holistic approach to care and conservation of natural history collections would be wise also. While some measures are very simple and inexpensive to do, other directives and recommendations for special equipment; storage systems and storage materials given in the book are either too expensive or unavailable in some countries.

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To know what is the best practice helps us to carry out potential actions closer to the "ideal." This book is a great resource for conservators and I wish we would be able to have it translated to Spanish some day.—*Soledad Tancoff, Museo Paleontologico Egidio Feruglio, Av. Fontana, Trelew (U9100GYO), Pcia. Chubut, Argentina*

BOOK REVIEW

CONSERVATION TREATMENT METHODOLOGY, 2007, Barbara Appelbaum. (Butterworth & Heinemann (Elsevier), New York., 468 pp.) The author, a prominent objects conservator with over 35 yr of experience, sets out to describe those decisions that are common to all appropriate treatments. She succeeds in producing a book that outlines the processes that collection managers and curators, as well as conservators, should keep in mind when working with objects in their care.

The author says that this book developed out of a wish to determine what all ethical conservation treatments have in common. SPNHC has been addressing long-term preservation and conservation since its inception in 1985. However, most SPNHC members are not conservators and may not have considered that some of their daily collection actions may, in fact, be conservation treatments. Daily activities undertaken by collection managers and curators in natural history collections, including adjusting alcohol solutions and extracting fossils from matrix, removing dust, and adhering broken specimen blocks, have long-lasting consequences to the specimens. Collection managers and curators perform the same functions of discovery, preservation, maintenance and remediation that conservators in other museum fields undertake. Although this book is clearly aimed at an audience of conservators, all practitioners in natural history collections, and therefore the individual objects and specimens under their stewardship, would benefit from an understanding of conservation theory and the decision-making methodology outlined in this book.

With relatively few available books, one such as this which so clearly explains conservation methodology is not to be overlooked. Other books exist but they usually deal with the theory of conservation, or specific treatment decisions. *Conservation Treatment Methodology* is not a technical manual that describes specific treatment techniques as is common in existing conservation literature, but rather a compendium of the collective knowledge that conservators have managed to pass to the next generation without writing it down until now.

Section 1 involves characterizing the object. The main point here is the recognition of object-specific attributes, material science and aging characteristics, object history and value, and the object's place in our world. I found the most fascinating part of this section to be a discussion of the relevance of assigned values to the treatment of an object. The values considered are: art, aesthetic, historical, use, research, educational, age, newness, sentimental, monetary, associative, commemorative, and rarity. Embedded in this list of values is nearly every reason for which museums collect. In natural history, the tendency is to consider only research value, yet popular inspiring exhibits demonstrate other values appreciated by the public. Recognition of value usually falls to the curator, rather than to the conservator, still these values play an important role in the determination of appropriate treatment goals.

Section 2 deals with the establishment of treatment goals. You mentally recreate the object from inception to now, and choose one point in its lifespan that is the ''ideal state'' to recreate. Appelbaum argues, fairly convincingly, that an appropriate treatment goal is always a point in the object's history. For example, a dusty basket with a combination of native (pre-acquisition) and heavy-handed recent but intact

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repairs is brought to the conservation lab for treatment. Using Appelbaum's approach, the curator and conservator would decide on an appropriate moment in the object's history, and try to recreate that physical condition rather than developing a checklist of tasks (reduce dust, disguise the ugly repair, etc.). For our basket, the preferred state may be the condition upon acquisition by the museum; the native repairs prove use and also retain historic, research, educational, and associative values so they will be kept as is, but we will reduce the collected dust, remove the ugly recent repair, and re-hydrate the basketry fibers to improve resiliency and discretely reweave the basket.

This approach can be easily used with historic and cultural collections, which are man-made items. Specimens documenting the world's biota (e. g., snails, plants, fish) can use the time when first prepared as an origination point. In regard to paleontological specimens, for example, often preservation itself changes the nature of the object, and it cannot be changed back to say, a point just after death. A museum's fossil is in a state that it had never been in prior to its collection and lab preparation. It simply cannot be returned to prior state, nor would that be desired. In any case, the ideal previous state approach is a good exercise, even if not always possible, and when combined with actually achievable results it will help direct the conservator and curator to an appropriate, realistic, and ethical goal.

Sections 3 and 4 are most typical of conservation literature. In section 3 the aspects that contribute to informed decision-making in regards to treatment materials and methods are discussed thoroughly and eloquently. Discussing materials theoretically (you'll find no recipes here), Appelbaum makes the important but often under-explored point that ''chemical stability is what makes a material 'conservation quality', but material choice for a particular treatment involves other considerations.'' The same could be said for treatment method. There are many good options available but all choices of materials and methods must be justifiable. Each object has a unique combination of characteristics and many factors, including stability, application technique, compatibility, future use and environment, must be considered when making treatment decisions.

Section 4 discusses how to make conservation documentation user-friendly. Conservation documentation is often a wealth of information that only confuses and frustrates owners and curators. Rather than recommending the inclusion of all imaginable data in the documentation, as I was encouraged to do in graduate school, Appelbaum says to minimize and tailor information to that which is actually of interest to the custodian. Documentation has always been the hallmark of responsible and ethical conservators, but it is often discarded by owners, and signed but unread by curators. It can be a fabulous resource but only if it is read and understood. By doing the research to make treatment decisions, and writing a proposal that is non-technical, both the conservator's need for documentation, and the custodian's need for clarity are met. This may be the most practical advice that conservators can glean from reading this book, and curators, collection managers, and technicians should appreciate and internalize: clear documentation is important.

The author is successful at formulating a methodology of decision-making. She makes it far less intimidating than it sounds: really just putting into words that which most conservators do instinctively, but often have difficulty expressing to curators, clients, and other custodians with whom we work. It was enjoyable, as a conservator, to read this book; this is a much more articulate version of my stream-of-

consciousness. One colleague said that reading it was like going to therapy. This will become an essential text for student conservators, and will be helpful to working conservators and to everyone who is responsible for collections care.—*Victoria Book, Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, 2401 Chautauqua Avenue, Norman, OK 73072*